Discovery of a Selfish Supergene's Dispersal Phenotype in House Mice *Mus musculus domesticus*

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> *There is grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one; and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved.*

– Charles Darwin, *On the Origin of Species*

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Summary

The organism and its genome are a remarkable cooperative achievement of billions of DNA bases that work together. Natural selection has shaped the genome into cooperation by generally favoring those genomes that work well as a whole, rather than resembling collections of genes that do not produce anything greater than the sum of their parts. But what seems perfectly harmonious is actually the site of an ever-lasting struggle over transmission from one generation to the next. This struggle is only contained by the benefits that cooperation accrues for each genetic element. In organisms with two sets of chromosomes, each gene is only transmitted to half of all offspring, but from the perspective of the gene, it would be much preferable if it was transmitted to all. Consequently, some genes manipulate that process and increase their own transmission to the detriment of the genes that are thereby transmitted less often as well as the rest of the genome. The *t* haplotype in house mice is such a selfish actor in the genome of house mice that carry it. It is a collection of linked genes, a supergene, that makes up 1% of the house mouse genome. It increases its own transmission from male carriers to their offspring to over 90%, rather than the expected 50%. However, not every mouse carries the *t* supergene, which puzzled biologists given its increased rate of transmission. This is due to the *t*'s two strongly disadvantageous traits. First, mice that carry the *t* haplotype on both chromosomes are either infertile as males or completely inviable. This immediately puts an upper limit on the frequency of the *t* haplotype in any population, because at a minimum not all mice can carry it on both chromosomes. However, this disadvantage alone would still allow for high frequencies of the *t*, but that is not what is found in nature. The second disadvantage of the *t* is likely a consequence of the mechanism with which it increases its own transmission. The *t* increases its transmission using a poison-antidote mechanism; it poisons all sperm of its carrier, but there is an antidote in the sperm that carry the *t*, thus only sperm that do not carry the *t* should be harmed. However,

t-carrying sperm are negatively impacted by this poison-antidote mechanism, as well, but to a lesser degree. The damage caused by this mechanism comes into full effect when *t*-carrying males are mating with females who also mate with other males in the same estrus cycle. This constellation creates competition between the sperm of the different males that mated with the female. In sperm competition, *t*-carrying males are much less successful in fertilizing the female than males who do not carry the *t*. This second disadvantage is so strong that it could explain the very low frequencies of the *t* in the wild. In very dense populations, where sperm competition is more common due to more matings per estrus cycle, the *t* can even go extinct, opening the question why the *t* has not gone extinct completely.

In this thesis, I am introducing, testing, and verifying the hypothesis that the *t* haplotype increases the probability with which *t*-carriers emigrate from populations to settle elsewhere, a process known as dispersal. Dispersal is a dangerous behavior, which is why the costs and benefits of it have shaped individual propensity to disperse over evolutionary time. Thus, a deviation from "normal" odds of dispersal could be against the interest of the organism as a whole. In Chapter 1, I introduce the reader to the broader picture of the conflict between genes within an individual's genome. In Chapter 2, I describe the hypothesis that *t*-carriers should be more dispersive than mice who do not carry the *t*, because this way the *t* is better equipped to avoid populations in which its disadvantageous traits are most pronounced. I tested this hypothesis using an intensively studied population of house mice and found an increased number of *t*-carrying mice emigrating from the population. In Chapter 3, I investigate the evolution of increased dispersal more formally using computer simulations. I find that the two disadvantageous traits of the *t*, inviability when carried on both chromosomes and poor performance in sperm competition with other males, indeed select for increased dispersal. However, the increased transmission alone is not sufficient to evolve increased dispersal. In Chapter 4, I verify the hypothesis using controlled experimental setups and I furthermore find that *t*-carriers are also heavier, more likely to disperse at higher weights, and more prone to explore unknown areas than mice who do not carry the *t*, which are all traits that could be beneficial for mice who are more likely to disperse. I conclude that the *t* haplotype appears to produce a remarkable dispersal phe-

notype in the mice that carry the *t*, which is a rare finding that should combine very well with the *t*'s increased transmission. Finally, in Chapter 5, I provide an outlook on work towards understanding the genetic basis of the *t*'s influence on dispersal. I describe a novel adaptation of a statistical method that allows us to gain insights into the genome sequences of mice much more cost-efficiently than what used to be possible, which will enable us to study the genetic basis of dispersal in house mice.

1 General Introduction

> *We are survival machines—robot vehicles blindly programmed to preserve the selfish molecules known as genes. This is a truth which still fills me with astonishment. Though I have known it for years, I never seem to get fully used to it.*

– Richard Dawkins, *The Selfish Gene*

1.1 The hidden conflict within life

Evolution is a remarkably simple process: whatever heritable traits an individual that is producing the most successful offspring possesses will become more common. This process of natural selection was discovered by Charles Darwin and Alfred Wallace (Darwin and Wallace 1858; Darwin 1859) at the end of the 19th century and has had remarkable success at explaining patterns in nature. However, while still broadly true, this theory came before the discovery of DNA as the primary means by which traits are encoded and transmitted to offspring (Koltzoff 1928; Griffith 1928). In the 20th century, biologists increasingly acknowledged that it is not so much the individual, but segments of DNA—especially genes—that are in fact the primary unit on which selection works (Hamilton 1964; Dawkins 1976). This can be translated into an updated description of selection: whatever gene is good at producing more copies of itself is going to be more common than others. But does this perspective really differ from the one that puts the individual in the focus? To discover this, we need to ask what makes a gene good at producing more copies of itself, or in other words what increases its "fitness".

A gene variety (an "allele") that increases the fitness of its organism will also increase its own fitness. For example, individuals are often found to be adapted

to local conditions, which becomes evident when they are displaced and suffer decreases in fitness (Hereford 2009). This difference in fitness can be caused by specific alleles. Take the alleles of the gene *SAG21*, which is involved in water stress tolerance in the model organism mouse-ear cress *Arabidopsis thaliana*. They affect the survival of the plant differently depending on the environment and they are consequently more or less common depending on the climate of the population's geographical location (Fournier-Level et al. 2011). In such a case, the fitness of the allele and the fitness of the organism are tightly linked.

But this obfuscates an important aspect: DNA is locked into perpetual conflict not just between individuals, but within organisms as well (Burt and Trivers 2006). Consider the situation in diploid organisms. If two parents have one offspring, each parent contributes 50% of their genome to the new genome. In general, every allele has therefore a 50% chance of being transmitted with each reproduction event. By simple stochastic laws, this means that with two offspring, an allele has a chance of 75% to have been passed on to at least one of them, but it would take seven offspring to reduce the chance of not being transmitted to below 1%. Seven offspring, let alone seven offspring who also manage to successfully reproduce, is not a guaranteed figure by any means. Thus, it would be beneficial for any allele to manipulate those numbers in its favor. And yet, most alleles do not do that. Instead, they are adhering to the unwritten rule of "Mendelian segregation", meaning they are transmitted at a rate that cannot be distinguished from random, i.e. at precisely 50%, because the rest of the genome selects against those that deviate from this rule (Crow 1991).

1.2 What are selfish genetic elements?

However, some DNA segments nonetheless break this rule. They are known as selfish genetic elements (Burt and Trivers 2006) and increase their own frequency against the interest of the organism and the rest of the genome. There are two broad categories of selfish genetic elements. The first category consists of selfish genetic elements that are increasing their frequency within an organism by making extra copies of themselves. For example, transposable elements copy themselves to other regions in the genome and B chromosomes

are additional chromosomes that are not necessary for the individual and exist because they employ mechanisms to nonetheless remain in the genome. The second category is made up of elements that increase the rate at which they are transmitted to the next generation without changing their frequency within an individual. The primary example for such elements are meiotic drivers.

Meiotic drivers manipulate the process of meiosis to increase their presence in the gametes of an organism (Núñez et al. 2018). They have typically been found in the best-studied model organisms, within diverse clades, from unicellular fission yeast with *wtf* genes (López Hernández and Zanders 2018) over *Drosophila* fruit flies (Jaenike 1999) to house mice (Chesley and Dunn 1936), which is testament to the idea that they may be much more common than the only the ones we know because they are hard to detect, but were found where we looked carefully. Thus, these extreme outcomes of within-genome conflict may be very common indeed. They work by either targeting gametes carrying an allele that is only on the chromosome that does not carry the driver or by damaging all gametes, but rescuing the gametes that carry the driver using an antidote.

Meiotic drivers are generally expected to meet one of two fates (Price et al. 2019). The first fate occurs if they are very efficient and subsequently spread to fixation. In this case, they would become "invisible" to researchers because they would in fact not drive anymore, because drive requires a competing chromosome to drive against. Fixated drivers would therefore not be detected, unless they are only fixated in one or some populations. In such a case, as is for example observed in *Drosophila*, drivers can be detected in inter-population crosses (Tao et al. 2001). The second fate concerns the opposite outcome: if drivers are not spreading fast enough, then the rest of the genome has time to adapt and evolve suppression of drive. In this case, the driver would die out and disappear. Therefore, in either case, we should usually not detect drivers, but yet we do.

What is different about the drivers that have been discovered? Why are they not fixated or extinct (Lindholm et al. 2016; Price et al. 2019)? The simplest explanation could be that they are somewhere in between fate one and fate two and will eventually arrive at either. However, some drivers are millions of years old and should have had enough time to fixate or be fully suppressed. Another

possibility is that the drivers have been in a long arms race with suppressors, during which they became so complicated that it became less and less likely for a successful suppressor to evolve (Price et al. 2019), which is difficult to test as there are few systems to compare. Are we missing undiscovered traits that could enable such persistent survival?

1.3 The *t* haplotype, a selfish genetic element in house mice

In this thesis, I am focusing on the *t* haplotype, a meiotic driver in house mice *Mus musculus*. It comprises the proximal third of chromosome 17 and is consequently circa 35 megabases or 1% of the mouse genome in size. It consists of at least four major, non-overlapping inversions that effectively reduce the recombination rate with the homologus chromosome to almost zero (Silver 1993; Kelemen and Vicoso 2018), thereby keeping the *t* haplotype intact when transmitted and allowing selection to work on the *t* as a whole. Male heterozygous carriers of the *t* haplotype (notation: +/*t* or *t*-carriers) transmit the *t* to more than 90% of their offspring (in contrast to the expected 50% in diploid organisms). Female +/*t* transmit the *t* at regular rates (50%). Therefore, the *t* only drives in males. It achieves this by manipulating spermatogenesis to reduce the motility of all sperm, but at the same time it provides an antidote for *t*-carrying sperm only (Herrmann and Bauer 2012). The drive mechanism involves multiple loci within the *t* haplotype region, which is why the reduction of recombination is essential for the *t*'s drive. However, the *t* loses out on the benefits of recombination, particularly the increased selection against harmful mutations, as reduced recombination is expected to lead to an accumulation of harmful mutations (Rice 2002). Consequently, the *t* haplotype has been found to carry harmful mutations and is either lethal in homozygotes (Klein et al. 1984) or makes male *t*/*t* infertile (Lyon 1986), with the former being the case in the population that I studied

Given these traits, advantageous and disadvantageous, of the *t* haploype, theory predicted that the *t* should be found at high frequencies in wild populations (Bruck 1957). However, this was never found to be the case (Ardlie and Sil-

ver 1998). This unexplained deviance between theory and reality was coined the "*t* paradox". Decades later, another trait of the *t* haplotype was discovered that would explain this paradox: During spermatogenesis, when the *t* harms all sperm, but rescues the function of *t* sperm, the *t* inadvertently also harms *t* sperm because the antidote does not fully rescue their motility (Burt and Trivers 2006). As a consequence, when females mate with multiple males in one estrus cycle and the males's sperm competes over fertilization, +/*t* males sire almost none of the offspring (Sutter and Lindholm 2015). This effect is so strong that it can lead to local extinction of the *t* haplotype, as seen in one study population that we also analzye in Chapter 2 (Manser et al. 2011). In a sense, this represents suppression of the *t* haplotype, although there is no evidence that female multiple mating only exists or is increased in house mice because of the *t* haplotype (Manser 2015), in contrast to *Drosophila* where rates of female multiple mating increased in the presence of a driver (Price et al. 2008; Wedell 2013).

1.4 Why did the *t* haplotype not go extinct?

The discovery of the *t*'s massive disadvantage in sperm competition may have explained the *t* paradox, but it has not fully illuminated *t*'s existence as a driver that is neither fixated nor extinct, but somewhere in between. It is now clear why the *t* did not fixate: it is highly deleterious in homozygotes and very unfit in female multiple mating. However, what is perhaps even less clear than before is why it did not go extinct, especially considering that the *t* is estimated to be two million years old (Silver 1993).

Anna K. Lindholm and I speculated that if a *t* haplotype variant could increase its odds of being present in populations that increase the effect of its drive and decrease the effect of homozygosity and sperm competition, then such a variant should be selected and eventually replace variants that do not achieve this. But how could such a variant do this?

One way individuals can optimize their fitness is via dispersal. Dispersal is the act of emigration with subsequent immigration into and breeding in a new population (Matthysen 2012) and is in part based on dispersal-determining alleles within an individual (Saastamoinen et al. 2018). Dispersal contributes to

gene flow between populations (Bohonak 1999), which facilitates the transmission of alleles to new locations. The benefits of dispersal are manifold and include decreased competition with kin (Hamilton and May 1977), lower odds of inbreeding (Gandon 1999; Perrin and Mazalov 1999), and a potentially much more suitable environment. However, dispersal has very high costs, particularly for those who fail to successfully disperse (Bonte et al. 2012). Therefore, the decision to disperse is a difficult weighing of costs and benefits, indirectly done via selection. The decision to disperse is often mediated by environmental cues, such as population density (Matthysen 2005), which is known to be linked to increased sperm competition in house mice (Dean et al. 2006; Firman and Simmons 2008).

In Chapter 2, we introduce the hypothesis that the *t* haplotype was selected to increase the dispersal of its carriers. We speculate that the *t*'s deleterious traits, the low fitness in sperm competition and the homozygous lethality, could increase the benefits gained from dispersing for *t* compared to the rest of the genome, which would put selection pressure on *t* to increase the dispersal propensity of its carrier. Using data from an intensively monitored long-term study on house mice (König et al. 2015), we show that juvenile mice carrying the *t* were more likely to disappear from the population, especially when the population was dense. Furthermore, they were also more likely to migrate within the population.

This finding is remarkable, because no manipulation of dispersal by a selfish genetic element was ever discovered before. While the evidence from the long-term study was certainly strong, it was still only one correlational study. Therefore, in Chapter 3, I present a formal investigation into the veracity of our dispersal hypothesis. Together with Hanna Kokko, we simulated the evolution of the *t* haplotype's influence on its carrier's dispersal propensity using individual-based models. The results were very clear: the disadvantage in sperm competition puts selection pressure on the *t* to increase dispersal out of populations with increased sperm competition (due to increased population density) and the homozygous lethality selects for slightly increased dispersal propensity as well. The meiotic drive alone did not select for increased dispersal.

With these two pieces of evidence, a correlational study and simulations, lining up, we still needed one more step to verify the result and we also wanted

to better understand what exactly *t* is changing in the behavior of its carriers. In Chapter 4, we describe controlled experiments with *t*-carriers and their wild-type conspecifics. We tested differences in dispersal using replicate enclosure populations of different densities and we tested, in the same animals, for differences in activity and exploration phenotypes. We found that +/*t* were more likely to disperse from the enclosures than +/+, that they were more explorative, that they weighed more, and surprisingly, that heavier +/*t* were particularly more likely to disperse than heavier +/+.

1.5 The *t* haplotype, a dispersal polymorphism in house mice

All of these findings combined led to a remarkable conclusion: the *t* haplotype is not just a meiotic driver with interesting advantages and disadvantages. It appears to increase its carrier's dispersal propensity together with traits that in other species have been found in individuals that were optimized for dispersal capabilities, such as increased weight with increased tendency to disperse of heavier individuals, and increased exploration tendencies. The only other example for this in mammals is found in the, more extreme, distinct dispersal morph in the naked mole-rat (O'Riain et al. 1996). In addition to that, the *t* is expected to increase in frequency much more rapidly than other alleles when immigrating into a population in which its disadvantageous traits are not expressed as strongly (Levin et al. 1969).

I end the thesis by providing an outlook towards upcoming investigations into the genomic basis of the dispersal manipulation in +/*t*. In Chapter 5, together with Barbara König and Andrés Bendesky, we introduce a novel method to observe the genetic variation of thousands of individuals at much lower costs than ever before. With this method, we aim to characterize the genomes of all mice of the long-term study analyzed in Chapter 2 and find genomic regions that are responsible for the increased dispersal of +/*t* and, potentially, regions that modulate or suppress this change in dispersal, which would be evidence of conflict over this trait.

All in all, this thesis provides the full account of all the evidence to date that

a selfish genetic element is modifying the dispersal phenotype of its carrier in its own interest. I hope that you will find this discovery as remarkable and stimulating as I did.

2 Carrying a selfish genetic element predicts increased migration propensity in free-living wild house mice

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2.1 Abstract

Life is built on cooperation between genes, which makes it vulnerable to parasitism. Selfish genetic elements that exploit this cooperation can achieve large fitness gains by increasing their transmission relative to the rest of the genome. This leads to counter-adaptations that generate unique selection pressures on the selfish genetic element. This arms race is similar to host-parasite co-evolution, as some multi-host parasites alter the host's behaviour to increase the chance of transmission to the next host. Here we ask if, similarly to these parasites, a selfish genetic element in house mice, the *t* haplotype, also manipulates host behaviour, specifically the host's migration propensity. Variants of the *t* that manipulate migration propensity could increase in fitness in a meta-population. We show that juvenile mice carrying the *t* haplotype were more likely to emigrate from and were more often found as migrants within a long-term free-living house mouse population. This result may have applied relevance as the *t* has been proposed as a basis for artificial gene drive systems for use in population control.

2.2 Introduction

The genes within a genome must work together to produce a viable organism, but their interests are not identical (Frank 2003). This causes conflict, because not all genes in an organism will be transmitted equally to the next generation. Consequently, a fair chance of transmission is necessary for cooperation within the genome over evolutionary time. Genes that violate this rule by increasing their chance of transmission can gain large fitness advantages at the cost of those that transmit in a Mendelian fashion (Burt and Trivers 2006). This leads to selection for selfish adaptations and, as a result, counter-adaptations to this selfishness, initiating an arms race between selfish genetic elements and the rest of the genome. This arms race is similar to the one between hosts and parasites, where some parasites even manipulate their hosts. For example, a parasite of the paper wasp *Polistes dominula*, manipulates the behaviour of its host through changes in gene expression (Geffre et al. 2017). Instead of behaving as a member of the "worker" caste, a parasitised female will behave more

like the nest-founding "gyne" caste. However, she will not actually found nests, but will instead transmit the parasite to other nests. Other manipulations have been observed, for example, in fungi-infected ants that climb vegetation and remain latched onto it post-mortem. The fungus will then produce spores, which disperse out of the dead ant's body (de Bekker et al. 2014).

Host defences against parasites and "parasitic" (Östergren 1945; Orgel and Crick 1980) selfish genetic elements range from behavioural changes to increased resistance in infected populations. For example, populations of the amphipod *Gammarus pulex* that are not naturally infected with the parasite *Pomphorhynchus laevis* are more sensitive to the parasite's manipulation than naturally infected populations (Franceschi et al. 2010). This is evidence of an arms race. A similar counter-adaptation to selfish genetic elements is the suppression of the drive mechanism. For example, in systems with X chromosome drive in *Drosophila*, which lead to the killing of Y-carrying sperm, some (Y) chromosomes suppress the drive, restoring production of sons (Mercot et al. 1995; Jaenike 1999, 2001; see Hatcher 2000 for a review). Behavioural adaptations are also evident, especially in mating preferences that reduce transmission of parasites or selfish genetic elements. In the woodlouse *Armadillidium vulgare*, males discriminate against "neo-females" infected with feminizing *Wolbachia* bacteria, another type of selfish genetic element (Moreau et al. 2001). Similarly, females discriminate against individuals carrying a selfish genetic element in stalk-eyed flies (Wilkinson et al. 1998).

Male meiotic drivers are selfish genetic elements that manipulate spermatogenesis to favour the sperm that carry them by harming the sperm that do not (Taylor and Ingvarsson 2003; Price and Wedell 2008). This is expected to decrease the competitiveness of a male carrying the meiotic driver by decreasing the number of viable sperm and potentially damaging the driver-carrying sperm as a by-product (Price and Wedell 2008; Sutter and Lindholm 2015). In consequence, driver-carrying individuals will perform worse (Wilkinson and Fry 2001; Champion de Crespigny and Wedell 2006) in sperm competition, in which sperm of different males compete over fertilization. Additionally, females evolve higher remating rates in response to the presence of a selfish genetic element in *Drosophila pseudoobscura*, which increases sperm competition and reduces the element's fitness (Price et al. 2008). Potentially, the driver carri-

ers might not sire a single offspring despite mating (Sutter and Lindholm 2015) and the driver could go locally extinct (Manser et al. 2011). Because of this strong disadvantage, females can be selected to increase sperm competition to decrease the risk of transmitting a driver to their offspring (Zeh and Zeh 1996; Price et al. 2008; Wedell 2013). In response, the driver could manipulate the male host's reproductive behaviour as may be the case in *Wolbachia*-infected *Drosophila* that show higher mating rates (Champion de Crespigny et al. 2006). Not much is otherwise known about how male meiotic drivers respond to this counter-adaptation that increases the risk of their extinction.

The *t* haplotype (*t*) is a male meiotic driver in the house mouse *Mus musculus*. It consists of a set of genes, making up about 1.5% or 40 Mb of the mouse genome, that are linked by inversions (Burt and Trivers 2006; Kelemen and Vicoso 2018) and distort Mendelian inheritance patterns so that 90 - 99% of the offspring inherit the *t* from a heterozygous sire (Silver 1985; Lindholm et al. 2013). It harms its host in at least two ways. The *t* carries recessive lethal alleles, so that *t*/*t* die prenatally (Safronova 2009; Sutter and Lindholm 2015). In addition, *t* heterozygous (+/*t*) males are very poor sperm competitors, siring only 11%-24% of offspring when mating with a female who also mates with a wildtype male in the same oestrus cycle (Sutter and Lindholm 2015; Manser et al. 2017). In house mice, sperm competition intensity varies between populations (Firman and Simmons 2008) and is higher in larger populations (Dean et al. 2006), so that fitness losses of +/*t* males from sperm competition are likely to vary with population demography. This is consistent with a negative association between population size and *t* frequency found in a trapping study (Ardlie and Silver 1998). In an intensively monitored free-living large house mouse population, the frequency of the *t* decreased significantly over 6 years until no +/*t* were left (Manser et al. 2011) while population size increased (König and Lindholm 2012). Experimental evidence shows that *t* frequency decline in this population is not linked to mate choice against the *t* haplotype (Manser et al. 2015; Sutter and Lindholm 2016) as found by Lenington et al.(Lenington et al. 1992) in another population, but is influenced by sperm competition (Manser et al. 2011; Sutter and Lindholm 2015).

The decline of the *t* in the population was even more rapid than a model based on sperm competition predicted (Manser et al. 2011). One additional contribut-

ing factor could be that +/*t* individuals are more likely to emigrate from the population than +/+. We will use the term "emigration" when we mean leaving the natal population (the first step of dispersal (Matthysen 2012)), "migration" when we mean leaving and entering another deme or population (Baker 1978), and "dispersal" when we mean migrating and then breeding. Early theoretical work predicted that increased dispersal rates should be beneficial for the *t* haplotype by preventing it from extinction due to drift and allowing it to increase in frequency rapidly when dispersing to a suitable population (Levin et al. 1969). In this view, a suitable population would be one that has no +/*t* in it, because the fitness of the *t* is frequency dependent, with lower fitness at high *t* frequencies (van Boven and Weissing 2001). This is due to negative fitness effects (up to homozygous lethality) of deleterious mutations on the *t* (Silver 1985). Combined with the more recent discovery of low sperm competitiveness, the most suitable population for the *t* would therefore be one with as few +/*t* and as little sperm competition as possible, which is expected in smaller populations (Dean et al. 2006). A *t* variant that is more likely to disperse to such a population should therefore be at a selective advantage compared to other variants.

We hypothesized that a *t* mutant that increases the migration propensity of its host generally would more often disperse to suitable populations and would thereby be selected. The increase in migration propensity could be a function of population density (i.e. +/*t* might only emigrate more than +/+ in dense populations where sperm competition is more common (Dean et al. 2006; Firman and Simmons 2008)). This has not yet been tested, but for parasites, theoretical work has demonstrated that they would benefit in general from manipulating their host's migration propensity (Boulinier et al. 2001; Lion et al. 2006). We analysed juvenile disappearances from and juvenile migration within an open population of wild house mice (the same as analysed for *t* frequency dynamics by Manser et al.(Manser et al. 2011)) to investigate if +/*t* individuals are more likely to disappear than +/+. We found that +/*t* juveniles were more likely to disappear from the population than +/+, particularly when juvenile densities were high. To our knowledge this is the first evidence of increased migration propensity of carriers of any selfish genetic element in a free-living population. Our research is particularly timely, as the *t* haplotype is proposed as a basis for artificial gene drive systems to eradicate house mouse populations (Backus

and Gross 2016; Piaggio et al. 2017) and behavioural differences in migration propensity between +/*t* and +/+ would need to be considered in modelling and implementing such systems.

2.3 Methods

2.3.1 The population

We analysed data that were collected between the years 2004 and 2012 in a free-living house mouse *Mus musculus domesticus* population in an old barn near Zurich, Switzerland (König et al. 2015). We provided a human-made and provisioned environment similar to that found in barns housing animals, but easier to monitor. We provided food and water regularly *ad libitum*. The barn is divided into four similarly sized sectors (König et al. 2015). However, mice can easily travel between these sectors and also freely enter and leave the barn. This emigration could not be monitored directly due to the numerous and unpredictable exit routes that mice use (that were however small enough to exclude predators). Instead, we used an indirect measure of emigration (see "Definitions of migration"). We considered individuals from 1 to 16 days as pups, then (when they begin to be weaned) as juveniles before reaching 17.5 grams in body mass, which is when we classified them as adults, as females do not breed until they exceed this body mass (König and Lindholm 2012). The sex ratio of the population was roughly equal (48% female).

2.3.1.1 Monitoring

When pups reached 13 days of age (allowing for ± 2 days of difference from this), they were ear-punched to provide a DNA sample. Every 10 to 13 days, the barn was searched for new litters. Every 7 weeks, on average, every individual in the barn was caught. On this occasion, all individuals above 17.5 grams in body mass received an RFID transponder and were then considered adults. On average in the years studied, 16.1% of the population received a transponder (was newly classified as an adult) on such a capture event. Additionally, we regularly searched the barn visually and with transponder scanners for dead individuals

or lost transponders. When found, dead individuals were removed and identified via their transponder or a new genetic sample. Finally, there is an automatic antenna system since 2007 in the population that tracks exits and entries of transpondered mice into and out of 40 nest boxes (König et al. 2015). We used these data in addition to data from manual checks to determine when an adult individual was last detected in the population if it was never found dead. This was relevant for the population size calculations, see "Controlling variables".

2.3.1.2 Identification

We genetically identify each individual as a pup, as a newly classified adult, or as a corpse if found dead without a transponder. We do so based on multi-locus genotypes based on 25 micro-satellite loci (Ferrari et al. 2019). The genotypes allow us to link individuals as pups to their adult transponder ID or to a corpse, allowing for one allelic mismatch using the software CERVUS (Kalinowski et al. 2007). We use the micro-satellite locus *Hba-ps4* that has a 16-bp *t* specific insertion (Hammer et al. 1989) to identify the *t* haplotype. Sexing of individuals was performed by testing for the presence of Y-chromosome-specific micro-satellite markers Y8, Y12, and Y21(Hardouin et al. 2010).

2.3.2 Definitions of migration

2.3.2.1 Disappearing from the population

Individuals that fulfilled all of the following criteria were classified as juveniles that disappeared from the population: 1) The individual was genotyped as a 13 ± 2 day old pup, 2) its genotype never matched to an adult's sample, and 3) also never to a corpse's sample. Following this definition, the time at which the individual disappeared must have been between 13 ± 2 days of age and an adult age (defined by body mass as described earlier) and therefore the individual was a juvenile. Consequently, individuals that disappeared from the barn as adults were not classified as disappeared in this analysis, but are instead treated as juveniles that stayed until adulthood. We excluded individuals born in the year 2005 from the analysis because monitoring was considerably less intense in this year and thus there is a larger potential to misclassify individuals that

died within the population as ones that disappeared. Therefore, we analysed 7 birthyears (2004 & 2006-2011) in which the *t* was present in the population (it then went extinct). We also excluded individuals about whom we did not have enough information (such as incomplete genotype or conflicting sex information) from the analysis. Furthermore, we removed those that died as juveniles, because we cannot know whether they might have emigrated later. Following these exclusions, 261 +/*t* and 2677 +/+ remained for the analysis (see S1 for an overview).

2.3.2.2 Migration within the population

We defined the four distinct sectors within the population described earlier as sub-populations between which mice can migrate. We did so, because from earlier analyses (Perony et al. 2012) we know that the dividing walls between the four sectors are social barriers for the mice. While mice are regularly seen moving within each sector, movements and social interactions between the sectors are less frequent (Perony et al. 2012). Furthermore, 61% of adults (in their adult lifetime) that were located at least 9 times were found within the same sector every time. 31% were found in two sectors in total, 7% in three, and less than 1% in all four. We defined juvenile within-population migrants as individuals that were first found as adults in a different sector than they were last seen in as pups. Thus, these individuals migrated in the same age range as those that disappeared. The dataset was based on the same restrictions made for the disappearance analysis, except that only those individuals that stayed in the population until adulthood could be analysed.

2.3.3 Controlling variables

Mice were counted towards the population size from birth until death or until they were last seen in the population. When they were last seen was based on both manually locating (in regular population monitoring) the animal or information from our automatic antenna system. A large proportion of the individuals disappeared from our population before they receive their RFID transponder (the disappearances analysed in this study). These mice were counted for 30 days from the time of their birth on as part of the population. This cut-off

is based on a handful of individuals that reached the body mass we designate as minimum for the transponder (17.5 g) at 35 days of age, reports of an early dispersal phase in 30 day old juveniles (Lidicker Jr. 1976), and a weaning age (nutritional independence and end of active maternal care) in mice of about 23 days (Bronson 1979; König and Markl 1987). Therefore, it is a conservative estimate of the minimum amount of time an emigrant would spend in the population after birth. However, the results of this study do not change fundamentally when this time frame is increased (we used 50 days of age as an alternative cut-off, see S1).

We subdivided the population size into adult and juvenile population sizes. We did so because we do not know how individual mice decide whether they migrate, therefore we wanted to disentangle the current and the future reproductive environment reflected by these two variables. The two population sizes are correlated, but do not explain much of variation of each other (linear model with $R^2 = 0.08$). Individuals that remained in the population until adulthood were counted from age 31 days on as part of the adult population (and before as juveniles), whereas individuals that were never found as adults were only counted for 30 days as juveniles and never as adults. We also considered using local adult population sizes in the four sectors, but overall did not find that to be more informative for the questions asked here (see S2). Similarly, we tested whether controlling for relatedness would influence the results, but concluded that this was not the case (see S3).

We defined the months April to September as the main breeding season, because these are the 6 months with the highest counts of new pups. The remaining months (October to March) were defined as the off-season. 87% of the birth dates in our dataset fall within the main breeding season. To account for inter-annual variation in the environment (like temperature or noises in the area) that could possibly affect migration propensity, we added the year of birth ($N = 7$) as a random effect in the disappearance models. Finally, we also controlled for the age when individuals were first sampled (between 11 and 15 days of age with most being sampled at 13 days). We did so because preliminary data visualisations revealed a relation between this age and disappearances.

2.3.4 Statistical analyses

2.3.4.1 Disappearing from the population

We utilised a generalized mixed effect model with a binomial distribution, a logit-link function, and fit by maximum likelihood. All statistical analyses and figures were done in *R* 3.4.4 (R Core Team 2018) with *RStudio* (RStudio Team 2016) and the packages *ggplot 2* 2.2.1 (Wickham 2009), and *lme4* 1.1-17 (Bates et al. 2015), the latter using the function *glmer*. The dependent variable was binary (1 when the individual disappeared as a juvenile and 0 if it did not). The independent variables were adult and juvenile population size (each standardized and fitted as linear and quadratic terms), the season, the sex, and the genotype. The population sizes and the season were taken from 30 days after an individual's birth to reflect the environment that the juvenile was exposed to around the time when it either did or did not emigrate. The year of birth was used as a random effect. We used *predictInterval* of *merTools* 0.3.0 (Knowles and Frederick 2016) with its integrated bootstrapping method with 10,000 simulations, using the median and a confidence interval of 95% for Figure 2.1.

We used *pbkrtest* 0.4-7 (Halekoh and Højsgaard 2014) for parametric bootstrapping based model comparisons with a significance level of 5%. Each dataset was simulated 10,000 times. The p-value is based on the PB statistic provided by the function *PBmodcomp*. It represents the fraction of likelihood ratio test (LRT) values of the simulated (bootstrapped) datasets that were larger or equal to the observed LRT value. Some of the runs can result in negative values of the LRT statistic. These runs are excluded automatically. We tested the significance of the genotype's effect and the interaction between genotype and the population sizes by comparing a model with to a model without the respective predictors (see Table 2.1 and S1 for all comparisons). We list ΔAIC values in the table to ease understanding, but did not use them for interpretation. We tested interactions of genotype and season as well as genotype and sex to explore potential relationships that we did not hypothesize (S1).

To test whether pup condition differences could be an alternative explanation for the disappearance differences, we used the same environmental variables to set up a linear mixed model that predicts pup body mass and then compared this model to one that also included the genotype as an effect (S4). We then

added pup body mass as a predictor to our disappearance null model and our most informative disappearances model (S1) to test whether a) disappearance is predicted by pup body mass and b) the genotype explains the same variation as does the pup body mass. All analyses that included body mass are reduced in their sample size by 40 individuals for whom we did not have this information.

2.3.4.2 Migration within the population

For this analysis, we have a reduced sample size because only mice that stayed alive and remained within the population until adulthood can be analysed. We also excluded one more birthyear because in 2011 no +/*t* stayed in the population until adulthood. We analysed 873 mice. The number of +/*t* in this dataset is small (60), which complicates statistical analyses. We compared the numbers of juvenile migrants between the genotypes with Pearson's χ^2 test using R. We also used generalized linear models to control for the same variables as in the disappearance analysis. The smaller sample size made this approach less informative. These results can be found in S5.

2.4 Results

2.4.1 Disappearances from the population

56% of all individuals born ($N = 2938$) in the years of this analysis who were alive shortly before weaning disappeared (Overview in S1). The most informative disappearance model included the genotype and an interaction between the genotype and the juvenile population size (model 2, see Table 2.1 and S1). This model indicated that +/*t* were more likely to disappear, particularly with increasing numbers of juveniles in the population (Figure 2.1). At mean juvenile densities, the probability that a +/*t* juvenile disappears was 47.5% higher than the probability for a +/+ juvenile (based on model predictions used for Figure 2.1). A standard deviation increase in juvenile population size increased this difference by 13.3 percentage points. As can be seen in Figure 2.1, +/*t* and +/+ were similar in their probability to disappear when there were few juveniles in the population, but then diverged with increasing juvenile density. Disappear-

Table 2.1: Excerpt overview of models of juvenile disappearances out of the study population (see S1 for the full table). LRT indicates the likelihood ratio test statistic of the observed dataset. The p-value is the fraction of simulated datasets with LRT larger than the observed LRT. Runs indicate the absolute values on which the p is based. The superscripted '2' in the formula refers to quadratic terms. The 'x' indicates model term interactions.

Models	Formula	Comparison	LRT	p	Runs	ΔAIC
Null model with covariates	∼ juvenile pop. size + juvenile pop. size^2 + adult pop. size + adult pop. size^2 + season + sex + age when sampled	NA	NA	NA	NA	NA
Model 1	∼ genotype + null model variables	Null model	16.00	0.0003	$1/5869$	-14.0
Model 2	∼ genotype x juv. pop. size + genotype x juv. pop. size^2 + model 1 variables	Model 1	11.62	0.005	$26/5815$	-7.62

ance probability decreased with increasing adult population sizes, but was not differently affected in +/+ and +/*t*. Similarly, being born in the main breeding season and being female increased the probability of disappearance for both genotypes (S1).

To test possible alternative explanations (other than migration propensity) for the disappearance probability of +/*t* (like a mortality or condition bias), we analysed data on dead juveniles found in the same time frame. We analysed data on 218 dead juveniles. We compared the number of dead juveniles with the number of individuals were found alive as adults between +/+ and +/*t* and found

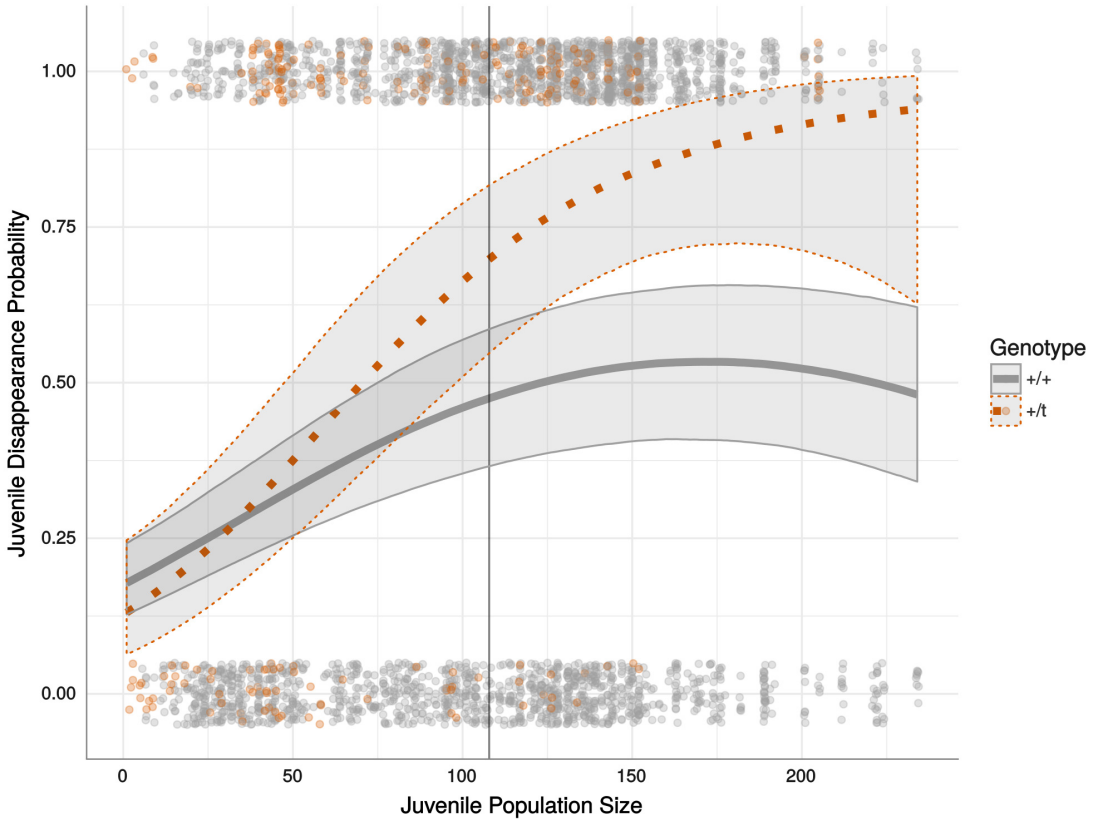

Figure 2.1: Predicted probabilities of juvenile disappearance out of the study population (lines) with 95% confidence intervals and actual data points (top and bottom, jittered) of +/t (orange, dotted line) and +/+ (grey, solid line) individuals in varying juvenile population sizes ($N = 2938$). This exemplary plot is based on predictions from the most informative disappearances model (model 2) for a female born in the off-season in average adult population size for no specific birthyear (fixed effects only). The vertical line indicates the mean juvenile population size.

no difference (+/*t*: 17.8% of 90 died as juveniles, +/+: 14.2% of 1424, $\chi^2 = 0.62$, $p = 0.43$). We decided not to conduct a more detailed model for this comparison because of the limited amount of juvenile +/*t* corpses found (16). For better comparison of this simple mortality analysis with the disappearance model, we used the same simple statistical test for the disappearance data used in the model and again found the difference between +/*t* and +/+ (71.6% of 261 +/*t* and 54.4% of 2677 +/+ disappeared as juveniles, $\chi^2 = 28.16$, $p = 1.1e-7$). We also tested whether there were any differences in the individual body mass as a pup (as a measure of the condition of the pup) between +/+ and +/*t*. We found that +/*t* pups were slightly heavier than +/+ pups ($\beta = 0.17g$, $p = 0.03$, intercept = 6.46g, details in S4), but did not find that the body mass as a pup predicts disappearances, either when the genotype was in the model or when it was absent (models 7 & 8, S1). Thus, we concluded that differences in juvenile disappearances between the genotypes cannot be explained by differences in juvenile mortality or condition.

2.4.2 Migration within the population

Of the 873 individuals analysed, 9.4% migrated as juveniles within the population, i.e. they were found in a different sub-population as adults than they were last seen in as pups. 16.8% of the 60 +/*t* migrated within the population as juveniles compared to 8.9% of 813 +/+, a statistically significant difference ($\chi^2 = 4.01$, $df = 1$, $p = 0.045$). Controlling for other explanatory variables in a GLM was more challenging due to the reduced sample size. We found overall that the genotype remained an informative predictor in interactions with juvenile population size and sex (comparison with null model: $p = 0.01$, $\Delta AIC = -7.22$, details in S5). Particularly, male +/*t* had a high migration propensity in the smallest population sizes.

2.5 Discussion

We provide evidence for a higher migration propensity of +/*t* juveniles compared to +/+ juveniles. We found that carrying the *t* haplotype is a strong positive predictor for juvenile disappearances out of our study population. Our hypoth-

esis that +/*t* should be selected to increase migration propensity was also modestly supported by a +/*t* bias in migratory movements within the population. Given that variation in behaviours related to dispersal is generally heritable to moderate degrees (Saastamoinen et al. 2018), a manipulation by the *t* in the *t*'s favour is a probable explanation. Our results further suggest that the rates of +/*t* disappearances are increased particularly in denser populations. This is consistent with previous results because the *t* was found to be less fit in denser populations due to an increase in sperm competition (Dean et al. 2006; Manser et al. 2011). The +/*t* that did not disappear from the population were found to be more likely to migrate within the population when juvenile densities were low. A possible explanation for this could be that there was more open habitat available when fewer juveniles were in the population and the migration-prone +/*t* were able to migrate within the population instead of needing to leave it.

We did not find a different effect of sex between the genotypes in our disappearance analysis, but did find one in the within-population migration analysis. The lack of difference agrees with a theoretical model that showed that *t* migration propensity manipulation need not be biased towards males (in which *t* drives), because migration of both male and female +/*t* was found to be more effective than male-only migration (Levin et al. 1969). However, +/*t* males were more likely than females to migrate within the population as juveniles. The test of this interaction was exploratory and not driven by a hypothesis. The result may reflect sex-specific costs and benefits of within-population migration for +/*t* mice, which would have yet to be fully elucidated. It is interesting, but needs further verification, particularly given that the disappearance analysis with its larger dataset does not show this pattern.

One drawback of our disappearance analysis is that it is at best an indirect measure of emigration, which we expect to be less precise. Despite that, we detected a strong signal. We considered alternative explanations of the strong +/*t* disappearance bias. We tested for a difference in juvenile mortality, but did not find one, which is further supported by a lack of difference in pup survival until weaning from lab-bred mice taken from the same population (Lindholm et al. 2013). We found a slightly increased pup body mass for +/*t*, but showed that this was not predictive of the disappearances (S1) and migration events (S5). Furthermore, there is evidence from another lab study that +/*t* and +/+ from the same

study population do not differ in adult body mass (males and females) (Sutter and Lindholm 2015). Differences in social dominance could be another explanation for disappearance patterns. Studies looking at dominance either found less dominant +/*t* males (Carroll et al. 2004), more dominant +/*t* males (Lenington et al. 1996), or no difference in dominance between males and less dominant +/*t* females (Franks and Lenington 1986; Lenington et al. 1992). However, if dominance differences were the cause of our disappearance results, we might expect to see an informative interaction between sex and genotype. Furthermore, we know from previous analyses that +/*t* males do not differ in survival from +/+ but +/*t* females live longer than +/+ in our population (Manser et al. 2011). Survival can predict dominance in house mice (Franks and Lenington 1986) and thus there is no clear evidence that dominance differs between the genotypes in our population. Finally, the mice in our population could go on exploratory trips outside the barn. Some of the exploring mice could be preyed upon on their trips. In that case, our results would in part reflect differences in exploration propensity. However, studies in mammals indicate that individuals that are more likely to explore are also more likely to migrate (Krackow 2003; Hoset et al. 2011; Debeffe et al. 2013) and if that is true in the study population we would still measure migration propensity indirectly through exploration propensity. Alternatively, if +/*t* juveniles are somehow more likely to be preyed upon than +/+, it would cause them to disappear more often without necessarily an increased migration or exploration propensity. We cannot test this idea with the data that are available to us. However, we believe that this alternative explanation is weaker than the one we offer. The difference between the genotypes in disappearances is larger in denser populations. This is more consistent with a density-dependent migration propensity than with predation risk. Furthermore, we found evidence that +/*t* may also migrate differently within the population than +/+, which provides further support for a difference in migration propensity. We cannot completely rule out a difference in predation risk as an explanation, but we argue that it is less likely than differences in migration propensity.

Generally, an increased migration propensity of +/*t* could help to explain why the *t* continues to exist in nature despite its homozygous and heterozygous fitness costs due to recessive lethals and low sperm competitiveness. Compared

to a *t* variant that does not influence migration, variants of the *t* that increase migration propensity could have an increased chance of reaching or founding populations where there are few other +/*t* and polyandrous matings are less frequent. The *t* is expected to rapidly increase in frequency given such circumstances (Lewontin and Dunn 1960; Durand et al. 1997; Ardlie and Silver 1998; van Boven and Weissing 1999; Manser et al. 2011). Thus, it would likely out-compete *t* variants that did not affect migration. Competition between *t* variants is consistent with genetic evidence that a single *t* haplotype variant recently replaced previous variants in a sweep (Hammer and Silver 1993). We do not know how an increased migration propensity could be encoded within the *t* haplotype, but the *t* comprises several hundred genes that are protected from recombination (Silver 1985). Alternatively, instead of manipulation by the *t*, the increased migration propensity could also be an evolved response by the rest of the genome to the presence of the *t*, if increasing migration propensity is increasing the fitness of the rest of the genome when *t* is present. More work is needed to better understand this interesting dynamic.

Emigration is only the first step of successful dispersal. Emigrants also need to breed as an immigrant or founder, which is challenging for mice (Pocock et al. 2005). Unfortunately, there were too few +/*t* that migrated within the population for us to analyse their breeding success. However, Anderson et al. (Anderson et al. 1964) were able to "infect" an island population with the *t* haplotype by manually migrating +/*t*. Although the *t* was able to establish itself in the initial area over a period of a few years, it did not spread much across the island. For Pennycuik et al.(Pennycuik et al. 1978), introducing the *t* to an enclosure was more difficult. However, they managed to do so when there were open territories in the population. They also reported many of the +/*t* males and females migrating between sub-populations. However, the *t* was almost extinct two years later, at the end of the study. It is evident from these experiments that there will be many populations to which the *t* cannot disperse successfully. In our study population we have no evidence for immigration of any individuals (unpublished). This makes increased migration propensity counter-intuitive because the migration will often fail. Still, because not migrating is also not beneficial for the *t*, it makes migration attempts potentially even more necessary for the *t*'s fitness.

When house mice invade an island that has evolved without mammalian

predators, their presence can be very damaging to the ecosystem (Wanless et al. 2007; Angel et al. 2009; St Clair 2011). Recently, efforts are being made to use a modified *t* haplotype for potential eradication of such house mouse populations (Backus and Gross 2016; Piaggio et al. 2017; Kanavy and Serr 2017; Gemmell and Tompkins 2017). The t_{SRY} variant is a *t* haplotype that is synthetically combined with the male-determining gene *SRY*. Every $+/t_{SRY}$ individual is thus expected to be male. Due to the *t*'s transmission advantage, more than 90% of the offspring of a $+/t_{SRY}$ are then male, which could then drive populations extinct via lack of one sex (Hamilton 1967; Price et al. 2010; Backus and Gross 2016). So far, only some of the *t*'s characteristics have been explicitly considered in trying to facilitate the use of t_{SRY} to eradicate wild populations (Backus and Gross 2016). However, accounting for the entirety of the known attributes of the *t* is crucial to successfully predict how a a synthesized variant works in the field. Increased migration propensity would likely aid in the distribution of $+/t_{SRY}$ mice to target locations, but could also increase the possibility of t_{SRY} reaching populations it was not intended for.

2.5.1 Conclusion

We found that juvenile mice carrying the *t* haplotype were more likely to disappear from the population at high densities and were over-represented in migrants within the population. To our knowledge, this is the first evidence of a change in migration propensity that is linked to a selfish genetic element. Our results should be of broad interest. First, they have implications for research on other selfish genetic elements, considering low sperm competitiveness is expected in many male meiotic driver systems like the *t* (Wilkinson and Fry 2001; Taylor and Ingvarsson 2003; Atlan et al. 2004; Price and Wedell 2008; Price et al. 2008). Recessive deleterious alleles and therefore frequency-dependent fitness would also be expected in other meiotic drivers, because without negative fitness effects the driver would spread to fixation (Hurst et al. 1996; Lindholm et al. 2016). This would provide further advantages for migratory variants of these drivers. Similarly, parasites could also benefit from manipulating dispersal behaviour (Lion et al. 2006). Second, the recent work on artificial gene drive systems based on the *t* haplotype will benefit from incorporating as many traits

of the *t* as are available. A difference in migration propensity could have important implications for such a system. Third, a selfish genetic element affecting migration propensity could be an important finding for research on dispersal and migration in general. Dispersal attempts are risky (Bonte et al. 2012) and the different selective pressures for the *t* and similar elements could help to explain better when this behaviour – that often results in no fitness gains – is most beneficial. Therefore, arms races like the one studied here could be a causal mechanism driving the evolution of dispersal. We will further investigate this new direction in *t* haplotype research with theoretical and experimental approaches.

2.6 Acknowledgements

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2.8 Competing interests

The authors declare no competing interests.

2.9 Author contributions

The study was conceived and the manuscript written by JNR and AKL. The data were collected and the genetic analyses performed by AKL and colleagues. Statistical analyses were performed by JNR.

2.10 Data availability

The datasets supporting this article have been uploaded as part of the supplementary material (S6-7).

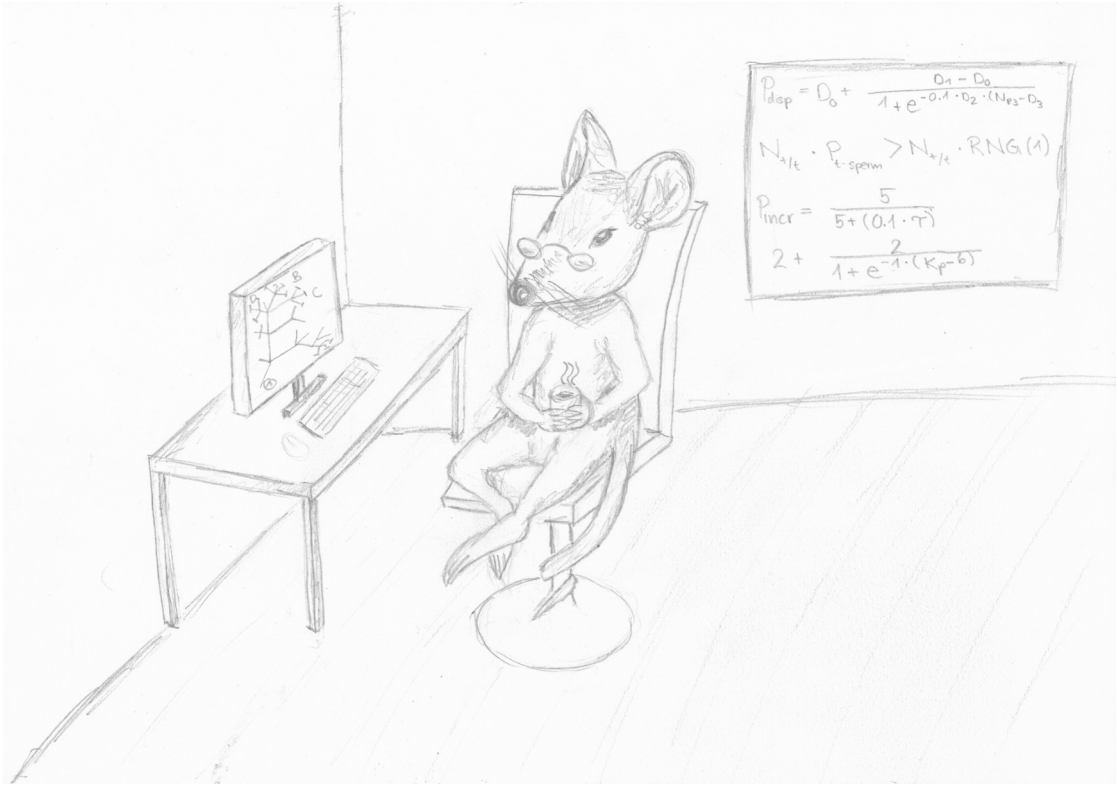
2.11 Ethics

The data were collected under permits 26/2002, 210/2003, 215/2006, 51/2010 from the Swiss Animal Experimentation Commission.

2.12 Supplementary material

Please find all supplementary material online in the published version of this chapter: https://royalsocietypublishing.org/doi/suppl/10.1098/rspb.2018.1333

3 Selfish migrants: How a meiotic driver is selected to increase dispersal



Germaine Léa Bongenge

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3.1 Abstract

Meiotic drivers are selfish genetic elements that manipulate meiosis to increase their transmission to the next generation to the detriment of the rest of the genome. The *t* haplotype in house mice is a naturally occurring meiotic driver with deleterious traits—poor fitness in polyandrous matings and homozygote inviability—that prevent its fixation. Recently, we discovered a novel effect of *t* in a long-term field study on free-living wild house mice: its carriers are more likely to disperse. Here we ask what known traits of the *t* haplotype can select for a difference in dispersal between *t*-carriers and wildtype mice. Our individual-based models placed loci determining a density-dependent dispersal function on *t* and the homologous wildtype chromosomes and tracked their evolution. The *t* haplotype consistently evolves to increase the dispersal propensity of its carriers, particularly at higher densities. By examining variants of the model that modify the presence of different costs caused by *t*, we show that the density-dependent effect is mainly driven by *t*'s disadvantage in polyandrous matings, while *t*'s lethal homozygosity can elevate dispersal somewhat across all densities. We also show aspects of intragenomic conflict in the co-evolution of wildtype alleles with their selfish counterparts.

3.2 Introduction

Conflict is everywhere. It not only takes place between species or between individuals, but also within individuals (Burt and Trivers 2006; Queller and Strassmann 2018). In general, all genetic elements are selected to increase the frequency at which they are copied to future generations. Most elements achieve this by increasing the fitness of the organism that carries them, which aligns the interests of the organism and its genome. However, there are also elements that increase their own representation in future generations at the expense of the rest of the genome, without a positive fitness effect on the organism. Since some even cause harm to the organism's fitness, these elements are known as selfish genetic elements (Burt and Trivers 2006). Selfish genetic elements come in a variety of forms. For example, killer meiotic drivers increase their frequency in the functioning gametes of an organism by inhibiting or destroying gametes

that do not carry the driver (Núñez et al. 2018).

Killer meiotic drivers work in one of two ways (Núñez et al. 2018): Either they release a killer element that attacks a target locus in *trans* (on the homologous chromosome), or they create a poison that attacks all meiotic products (indiscriminate of whether they carry the driver), together with an antidote that acts in *cis* and thus rescues only driver-carrying meiotic products. These poison-antidote drivers commonly work only in males and could suffer from reduced sperm competitiveness for reasons such as imperfect rescue and reduced gamete counts (Price and Wedell 2008). In such a scenario, the fitness outcomes for the driver differ dramatically between single matings and polyandrous matings. In the latter, where sperm from multiple males compete over fertilization, the driver-carrying poor sperm competitors are at a significant disadvantage.

Females commonly mate with multiple males in wild populations (Taylor et al. 2014). The frequency of polyandry has been linked to genetic and environmental factors, e.g. male fertility (Sutter et al. 2019), local density (Dean et al. 2006; Firman and Simmons 2008), and presence of meiotic drivers (Price et al. 2008). Less is known about meiotic drivers themselves adapting to local variation in polyandry: if drivers do better in single matings, can they somehow avoid ending up in polyandrous situations? One possibility is that drivers could increase the dispersal propensity of their carriers. This hypothesis is based on the argument that dispersal may lead to areas with less polyandry (via movement to less dense populations on average) and/or less frequent matings with another driver carrier (which causes some offspring to be homozygous for the driver, which is detrimental in the system that we study). We investigate these possibilities for a naturally occurring poison-antidote male meiotic driver in house mice (*Mus musculus*), the *t* haplotype, for which there is a wealth of knowledge of its traits.

The *t* haplotype comprises a 35 Mb linked region on an autosome, estimated to be two million years old (Silver 1993; Kelemen and Vicoso 2018). It manipulates spermatogenesis to increase its own chances of transmission (Lindholm et al. 2019). Heterozygous (notation: +/*t*) males transmit the *t* haplotype with 90% probability, leaving only 10% for the homologous wildtype chromosome (denoted +). This marked contrast with the "fair" Mendelian rate of transmission of 50% makes the *t* "selfish". However, despite this fitness advantage, the *t* does

not fix or persist at high frequencies in natural populations (Ardlie and Silver 1998). One reason is that homozygous (*t*/*t*) carriers of the *t* haplotype are either inviable (Klein et al. 1984) or sterile as males (Lyon 1986), which is a large cost to the *t*'s fitness (Dunn and Levene 1961; Safronova 2009; Sutter and Lindholm 2015). The *t* is however even less frequent in natural populations than would be predicted based on this trait (Bruck 1957; Ardlie and Silver 1998), a pattern known as the "*t* paradox" (Manser et al. 2011). This paradox was explained by another deleterious trait of the *t*: the sperm of *t*-carrying males (+/*t*), while almost exclusively transmitting the *t*, are less competitive than sperm of wildtype (+/+) males (Sutter and Lindholm 2015; Manser et al. 2017). As a consequence, +/*t* males sire a clear minority (11-24%) of the offspring of polyandrous matings when in competition with +/+ males.

An increased dispersal propensity conceivably improves the *t*'s chances of being present in multiple populations, new populations, and populations in which it is (temporarily) fitter than the wildtype (thus decreasing global extinction risk) (Levin et al. 1969; Hamilton and May 1977; Comins et al. 1980). In general, dispersal leaves more resources for related kin (Hamilton and May 1977) (in this case, other *t* alleles). This might not promote dispersal of *t* above that of the wildtype *per se*, since +/+ enjoy this benefit as well (likewise, arguments such as "being present in multiple populations is beneficial" apply to +/+ too), but for *t* there is a unique benefit of leaving a *t*-rich habitat patch. Their departure counteracts the possibility of two philopatric +/*t* individuals mating with each other and producing inviable *t*/*t* offspring. If dispersal of *t* brings its carrier to a population with a lower *t* frequency, the benefit occurs both at the new as well as the natal site.

As a flipside, however, entering dense, +-rich habitat patches induces a larger risk of losing out in sperm competition, because the frequency of polyandrous matings increases with population density in house mice (Dean et al. 2006; Firman and Simmons 2008). This makes us hypothesize that net selection on *t*-associated dispersal will depend on polyandry and on whether dispersal (on average) occurs from high density to low density sites. If it does, then the risk of competing with + sperm is alleviated, and we may then expect *t*-carriers to be particularly prone to leave high density sites (density-dependent dispersal). On the other hand, if the homozygous costs are larger than the polyandrous disad-

vantage, then we would expect +/*t* to disperse preferentially out of low density sites (where *t* frequency is expected to be high due to the effectiveness of the meiotic drive).

In this framework, *t* behaves somewhat like an infection, though for unique reasons (homozygote inviability). If *t* increases in frequency as a result of successfully entering new local populations, the relative fitness of *t* will decrease over time. Whether this selects for dispersal even out of low density habitats (with low multiple mating frequencies), depends on the balance of all the costs and benefits of dispersal. As a whole, we expect the costs and benefits of dispersal to differ between the wildtype and the *t* haplotype. Indeed, our previous empirical work on free-living wild house mice found that *t*-carrying juveniles were more likely to emigrate, and were over-represented in migration events (see Figure 3.1) (Runge and Lindholm 2018).

In this study we present results from individual-based models that simulate the evolution of the *t* haplotype's influence on its carrier's dispersal propensity. The results provide quantitative support for the hypothesis that *t* should evolve a density-dependent increased dispersal propensity. By considering multiple hypothetical scenarios, we find the *t*'s disadvantage in polyandrous contexts to be the main driver of its elevated and density-dependent dispersal propensity. While homozygous costs also play a role, they increase dispersal only modestly and independently of density.

3.3 The model

The model was written and executed in *NetLogo* 6.0.0 (Wilensky 1999) and we used *R* 3.5.1 (R Core Team 2018) with the packages *doParallel* (Microsoft Corporation and Weston 2018), *iterators* (Revolution Analytics and Weston 2018), *foreach* (Microsoft Corporation and Weston 2017), *stringr* (Wickham 2019), *readr* (Wickham et al. 2018), *dplyr* (Wickham et al. 2019), and *ggplot2* (Wickham 2009) for our analyzes and plots of the model.

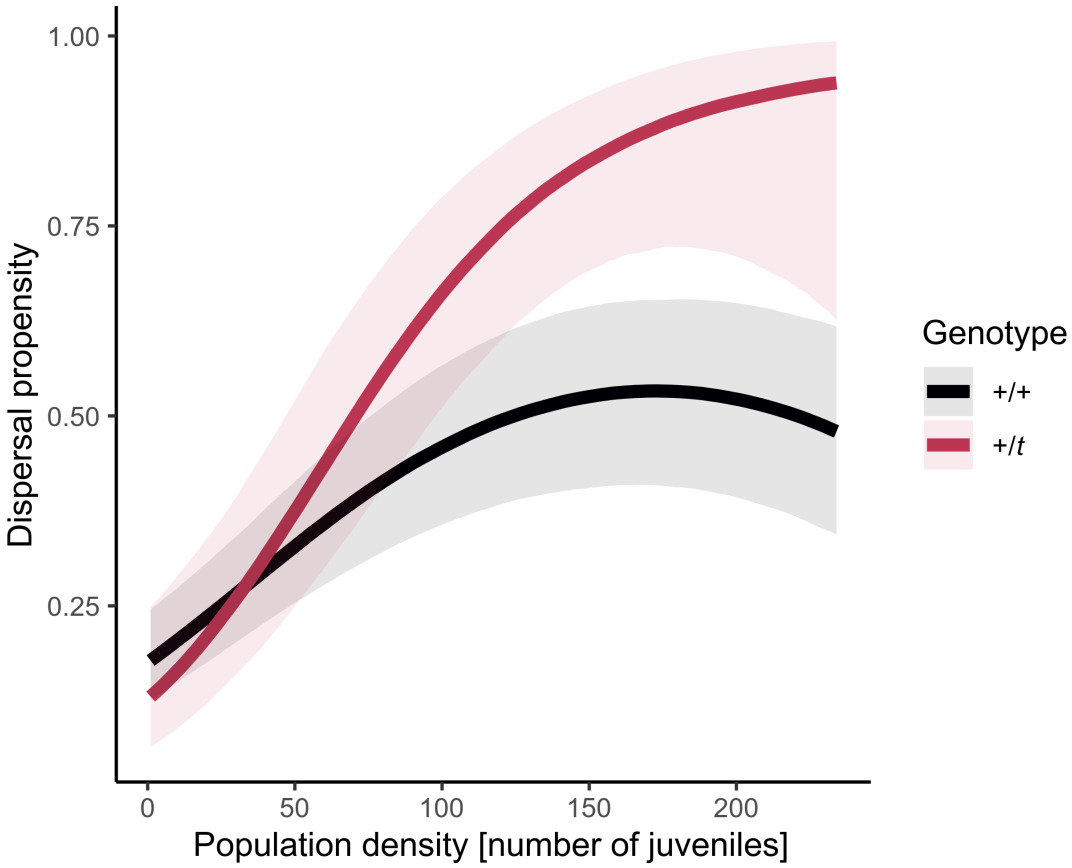


Figure 3.1: Differences in juvenile dispersal propensity between +/*t* and +/+ in a long-term field study, replotted from Runge & Lindholm (2018).

3.3.1 Purpose

The purpose of our individual-based model is to examine the evolution of the propensity to disperse, in a potentially density dependent manner, in a selfish haplotype (the *t* haplotype) and the homologous chromosome (here also modeled as a haplotype). We model the dynamics of the *t* and wildtype ("+") haplotypes based on known rules of meiosis and viability costs in the *t* system in house mice. We assume dispersal to follow a reaction norm based on four autosomal dispersal loci that are linked within the *t* or + haplotype. The simulation starts with the same density-independent dispersal propensity in all individuals (i.e. all reaction norms initially flat); mutations in dispersal loci are subsequently transmitted to offspring. Selection on dispersal is based on temporally fixed, but spatially heterogeneous local carrying capacities. Stochastic associations between local *t* frequencies and mating patterns combine to produce spatial and temporal variation in the relative fitness of *t* and +. Empirically, both +/*t* and +/+ mice are known to show density-dependent dispersal propensities (Runge and Lindholm 2018), while there is mixed evidence, but negative in our population, for female ability to distinguish between +/*t* and +/+ (Lenington 1991; Manser et al. 2015; Sutter and Lindholm 2016). We consequently assume that individuals can assess local density, but not the local frequency of the *t* haplotype or another individual's carrier status.

3.3.2 World

The simulated world consists of S^2 patches with wrapping boundaries (i.e. an individual can move past "the border" and will re-enter on the opposite side of the world). The patches are squares with sides of 1 unit length, and fill the entire space (e.g. a patch at coordinates $y = 0$ and $x = 0$ ranges from $x = -0.5$ to $x = 0.5$ and $y = -0.5$ to $y = 0.5$). Continuous space allows individuals to move in 360 degrees of direction. For certain interactions, we need data on all individuals within a radius R_x of the coordinates of a focal individual. We take the Euclidean distance (taking wrapped boundaries into account) to form the distances of *x* patch widths, which translates to counting mice in an area of $x^2\pi$ patches. Finally, each patch is assigned a K_p that determines its carrying capacity (for more details see *Mortality*).

3.3.3 The population

The model tracks the haplotypes of diploid individuals of differing sex, age, and location (overview: Table 3.1). An individual carries two homologous chromosomes. Each chromosome is a haplotype that comprises five linked loci. One locus determines whether the haplotype is + or *t*. Thus, an individual can be +/+ or +/*t*, with *t*/*t* being inviable. The other loci determine the dispersal phenotype (reaction norm) as described in *Dispersal*. An individual's age is the number of turns (see below) since birth. The location is indicated by x, y coordinates that can take any real number value, as individuals do not need to reside in the center of a patch. Simultaneously, each individual resides in a uniquely defined patch: For example, $x = 3.4$ and $y = 5.1$ assigns an individual to the patch that is centered at $x = 3$ and $y = 5$.

3.3.4 Turns

Within each turn, the following procedures are run for all individuals sequentially (i.e. every procedure is done for all individuals before the next procedure begins): movement, dispersal, mating, birth, and death together with aging of the survivors (see Figure 3.2). In other words, all individuals—in a random order—will perform the movement behavior sequentially (details described below). Once the last individual has finished moving, a new random sequence is drawn to determine the order in which individuals disperse (or not), according to their dispersal phenotype and age, followed by the next behavior in a new random order, until all behaviors are completed for this turn.

3.3.5 Behaviors

3.3.5.1 Movement towards the opposite sex

In this first part of a turn, a focal mouse moves towards one randomly chosen mouse of the opposite sex (a potential mate) within a radius R_1, assuming such a target mouse exists. If there is such a mouse, the focal individual will adopt the potential mate's coordinates and additionally, with a probability of P_{move}, move one patch width in a random direction. This additional movement is im-

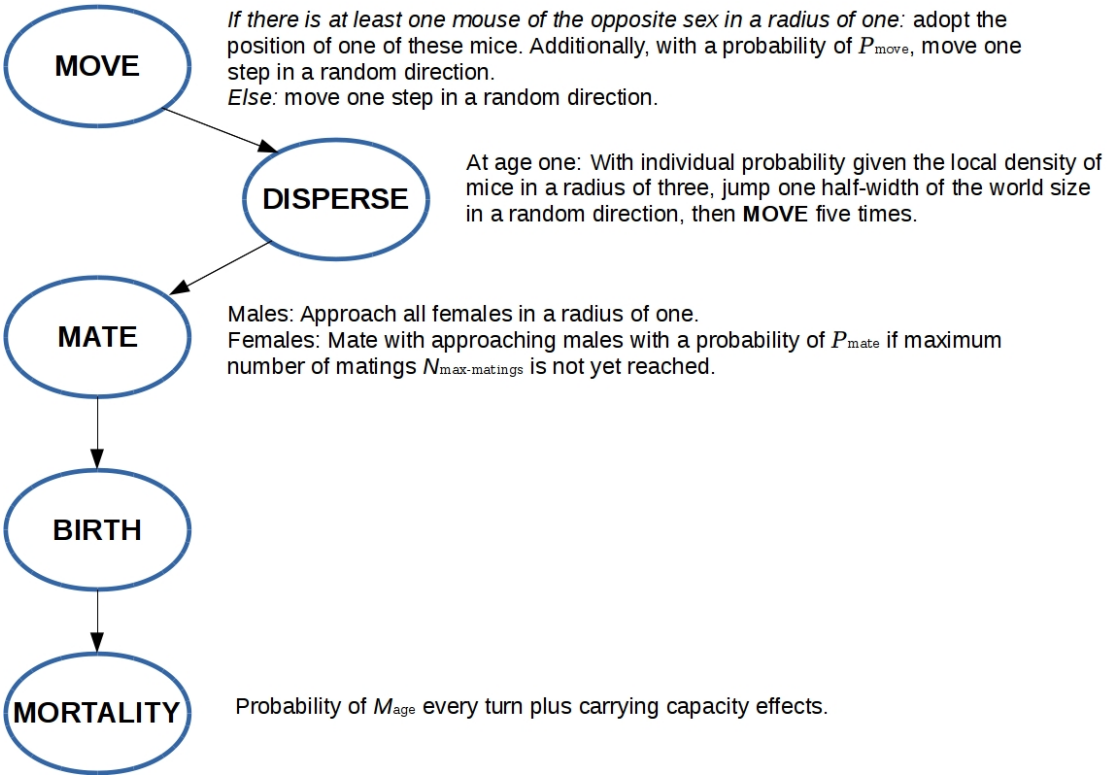


Figure 3.2: Overview of what happens during a turn from the perspective of a mouse in the model.

plemented to increase local density variation by having mice close to each other without all being on the same patch (and thus counting towards the same carrying capacity). If there is no mouse of the opposite sex, the focal mouse will always move one patch width in a random direction. These movements create spatial organization that resembles group-like structures, without requiring an explicit implementation of group membership.

3.3.5.2 Dispersal

The four loci D_0, D_1, D_2, and D_3 determine the dispersal phenotype, which is a reaction norm that relates local density to the probability of dispersal. D_0 represents the minimal dispersal propensity of the reaction norm, while D_1 is the maximum. D_2 is how steep the change in dispersal propensity is at the threshold local density D_3 (see Table 3.1). Individual dispersal occurs maximally once, as a juvenile (at age 1). The individual probability to disperse is given by P_{disp} (adapted from Gerber and Kokko (2018)).

(1)
$$P_{\text{disp}} = D_0 + \frac{D_1 - D_0}{1 + e^{-0.1 \cdot D_2 \cdot (N_{R_3} - D_3)}}$$

P_{disp} is a reaction norm that can increase or decrease (or stay unchanged) as a response to this measurement, depending on the allelic values at the four loci D_0 ... D_3. The values in equation (1) are the mean of both alleles for each locus, irrespective of whether the chromosomes carry *t* or +. R_3 is the radius around the focal mouse within which the local density N_{R_3} (the number of mice including the focal mouse) is measured, roughly equaling an area of 28 patches.

The mouse disperses if a random real number between 0 and 1 is smaller than P_{disp}. A dispersing focal mouse will first move a distance of $\frac{S}{2}$, i.e. half the world's width, in a randomly chosen direction, followed by executing the movement behavior (see *Movement towards the opposite sex* above) five times to decrease the odds of dispersing to a place where there are no mates. Dispersal is also costly, leading to death with probability M_{disp}.

Note that we impose boundaries that specify the range of permitted allelic values for each of the D_i. The only value that we constrain to be positive is D_2 as

this does not limit the number of possible phenotypes, but guarantees that phenotypes from mixing two dispersal functions (from two parents) make sense; if all D_i could take both positive and negative values, the same reaction norm could be achieved with different sign combinations, which then yields nonsensical combinations when sexual reproduction yields new mean D_i values. Since other D_i (than D_2) can be negative or above 1, P_{disp} may yield phenotypes that never or always disperse. Similarly, if the density threshold D_3 is higher than any density encountered or below 0, the reaction norm is flattened; we permit all this, as our goal is to allow many possible phenotypes to evolve (see Figure 3.3 for examples).

We refer to the dispersal propensity P_{disp} as the *genotypic* dispersal propensity, which we distinguish from the effective phenotypic range between 0 and 1 (*phenotypic* dispersal propensity). A genotypic dispersal propensity between -3 and 0 translates to a phenotypic dispersal propensity of 0, while a genotypic dispersal propensity between 1 and 3 translates to an phenotypic dispersal propensity of 1. Values between 0 and 1 are the same from both perspectives.

3.3.5.3 Mating

Mating behavior is initiated by males. During mating, a focal male approaches all female mice of age ≥ 1 in R_1. The approached female will mate with a probability of P_{mate} unless she has already mated $N_{\text{max-matings}}$ times in that turn. $N_{\text{max-matings}}$ is an individual variable of each female with two possible values, $N_{\text{max-matings}} = 3$ or $N_{\text{max-matings}} = 1$. The probability of the former, randomized for each female separately (and in each turn anew), equals P_{multi}, which is a parameter with which we adjust the global frequency of female multiple mating.

Note that a female that is receptive to mating multiple times still has to be approached by a male and choose to mate with him with P_{mate}. Thus, P_{multi} reflects the upper limit for the frequency of polyandrous females, and the realized frequency of multiple matings may fall below this value. This approach allows us to vary the frequency with which females mate multiply while keeping this frequency stochastic and heterogeneous throughout the simulation. Note further that while the number of times that females mate is capped ($N_{\text{max-matings}}$),

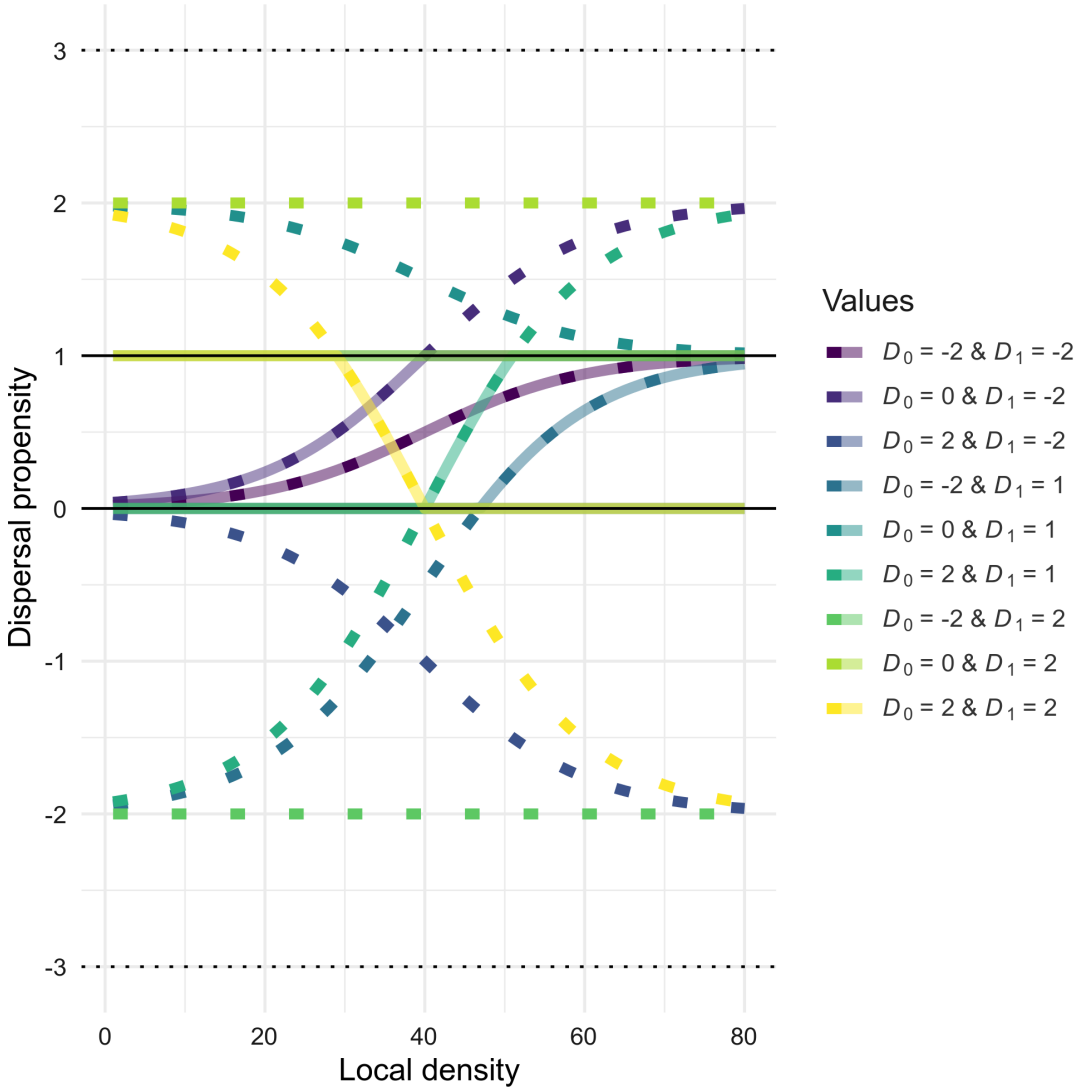


Figure 3.3: Examples of possible dispersal functions with varying minimum (D_0) and maximum (D_1) genotypic dispersal propensity, but constant steepness ($D_2 = 1$) and density threshold ($D_3 = 40$). The dotted part of each line indicates the genotypic dispersal propensity with parameter space from -3 to 3. The solid part of each the line indicates the phenotypic dispersal propensity between 0 and 1, with all genotypic dispersal propensities above 1 equaling 1 and all below 0 equaling 0.

it is unconstrained in males.

3.3.5.4 Birth

Each mated female begins her pregnancy with six offspring, with each offspring being assigned sex independently from each other (1:1 primary sex ratio); only viable (i.e. not *t*/*t*) ones will be born (aged 0). The sire for each young is determined independently with the following procedure. For mothers who mated singly, the sire is obvious. For mothers who mated multiply, the sire will be +/*t* if:

(2)
$$N_{+/t} \cdot P_{\text{t-sperm}} > N_{+/+} \cdot RNG(1)$$

where $RNG(1)$ is a random real number between 0 and 1, $N_{+/t}$ is the number of +/*t* males that mated with that female, $N_{+/+}$ is the corresponding number of +/+ males, and $P_{\text{t-sperm}}$ represents the probability of *t* sperm outcompeting + sperm (experiments (Sutter and Lindholm 2015; Manser et al. 2017) suggest $P_{\text{t-sperm}}$ between 0.11 and 0.24).

Within the appropriate genotype category of the sire (+/*t* or +/+), the actual sire is a randomly chosen male (no effects of mate order) among the $N_{+/t}$ or $N_{+/+}$ candidates. If the sire is +/*t*, the *t*-carrying chromosome is transmitted with probability P_{drive} (and the +-carrying chromosome with the complementary probability $1 - P_{\text{drive}}$). Females, as well as +/+ males transmit a randomly chosen chromosome. We do not make the chromosomes recombine, thus all loci contained by a chosen chromosome are transmitted to the next generation.

Finally, the dispersal loci D_i variables mutate in the offspring with a probability that is initially high (to allow for efficient searching of the space of possible reaction norms) and gradually diminishes. All dispersal loci mutate independently. We distinguish between incremental mutations and full mutations. Incremental mutations happen with a probability of

(3)
$$P_{\text{incr}} = \frac{5}{5 + (0.1 \cdot \tau)}$$

where τ is the turn number (see Appendix Figure 6.1). In case an incremental mutation takes place, the new value of the variable will be a random addition to or subtraction from the former value of up to 10% of the maximum parameter value (see Table 3.1). For example, if D_3 is limited to values between -100 and $+100$, an incremental mutation adds a uniformly distributed value between between -10 and $+10$. Incremental mutations that move the value outside the parameter space will set the value at the relevant boundary. A full mutation happens with probability $P_{\text{full}} = \frac{P_{\text{incr}}}{10}$ and changes the respective value to any randomly chosen value in the parameter space (in the example above any real number between -100 and $+100$).

3.3.5.5 Mortality

We include both density-independent and density-dependent mortality. A focal mouse dies due to density-independent causes with a probability of M_{age} per turn. After applying this mortality to all mice, we further impose patch-specific carrying capacities on the survivors, causing density-dependent mortality. In this procedure, we randomly iterate through all patches (rather than all mice). The carrying capacity of each patch is defined by

(4)
$$2 + \frac{2}{1 + e^{-1 \cdot (K_p - 6)}}$$

The carrying-capacity-determining variable K_p, which can be any integer between 1 and 10, is assigned randomly for each patch with a uniform distribution at the beginning of the simulation. If there are fewer mice on a patch than its carrying capacity, nothing happens. If there are more, only K_p will survive. The survivors are chosen randomly among the mice residing on the patch with e.g. a carrying capacity of 2.4 translating into 2 mice plus a 40% chance of one more mouse surviving on that patch in that turn (this chance is re-applied each turn). Finally, all survivors of density-independent and density-dependent mortality will increase in age by one.

Carrying capacities range from 2.01 to 3.96 mice. The values in formula (4) are chosen so that all patches offer chances of multiple mating (all carrying capacities are at least two mice plus a chance of one more), but on most patches

a significant possibility remains that only two mice (no multiple mating) survive in a turn. Since carrying capacities of patches are not spatially correlated, the environment is clearly heterogeneous for its potential for polyandrous matings.

3.3.6 Initialization

At the beginning of each simulation, N_{start} mice of age 1 are placed randomly into the world. Sex and genotype are distributed at random, with a probability of 0.5 for each genotype (+/+ and +/*t*) and sex, respectively. All mice start with all alleles of D_0, D_1, and D_2 loci at 0.5 (leading to a phenotype that disperses with probability 0.5; note that the consequent initial steepness $D_2 = 0.5$ has initially no effect as the dispersal rate is constrained to be between D_0 and D_1), and density threshold D_3 loci set to 0.

3.3.7 Conditions

We refer to the set of values $\{M_{\text{disp}} = 0.1, P_{\text{multi}} = 1.0$, and lethal *t* homozygotes$\}$ as the "natural condition" as it combines female multiple mating and fully lethal *t*/*t* with costly dispersal. Below, we describe the deviations from the natural condition that were also analyzed. All conditions were simulated with M_{disp} of 0.0, 0.05, and 0.1. At 0.15 (with lethal *t* homozygotes and $P_{\text{multi}} = 1.0$), *t* only survived in 32% of simulations ($n = 25$) until the 10,000th turn (in 0% at $M_{\text{disp}} = 0.2$, with $n = 25$), while it still survived in 100% at $M_{\text{disp}} = 0.1$ ($n = 25$). We decided not to further analyze $M_{\text{disp}} > 0.1$ for computational reasons.

3.3.7.1 Female multiple mating

To examine how polyandry impacts the divergence between evolving dispersal propensities in the + and *t*, we varied P_{multi}, the probability with which a female is receptive to mating multiply (see *Mating*), in increments of 0.025 between 0.0 and 1.0.

3.3.7.2 Homozygous lethality

To examine the extent to which *t*'s homozygous lethality is responsible for dispersal evolution is challenging, because *t* quickly fixates if it is not homozygous lethal (see Appendix Figure 6.3). The subsequent evolution of dispersal in the absence of genetic variation makes dispersal in *t*/*t* populations behave analogously to those in +/+ populations (except for noise, see Appendix Figures 6.12-6.13). To overcome this challenge, we created a condition aimed at reducing the homozygous cost of *t* while maintaining both *t* and + in the population. We simulated "semi-viable" homozygotes by birthing a +/*t* whenever an inviable *t*/*t* would have been born (i.e. when +/*t* ×+/*t* matings produce a dead *t*/*t*). This reduces the fitness cost of +/*t* ×+/*t* matings on the *t* while also preventing the *t* from fixating, enabling us to continue to compare it with the wildtype. The differences between these conditions were not as large (see below), and therefore we decided not to proceed to finer details of varying the cost of *t* homozygosity, and most of our results below instead focus on female multiple mating.

Table 3.1: Overview of simulation variables

Variable	Type	Value	Description
S	Global	60	The width and height of the world
N_{start}	Global	5,000	The number of starting mice
P_{drive}	Global	0.9	The transmission advantage of *t* sperm over + sperm *within* +/*t* males.
$P_{\text{t-sperm}}$	Global	0.19	The chance of a +/*t* sire in a female multiple mating with one +/*t* male and one +/+ male.
P_{multi}	Global	0.0 to 1.0	Chance that a female will mate multiple times per turn

Variable	Type	Value	Description
P_{move}	Global	0.5	Probability of moving even after finding an opposite sex partner
P_{mate}	Global	0.75	Probability of a female mating with a male that approaches her if she is not at her mating limit yet.
M_{disp}	Global	0.0 to 0.1	Dispersal mortality
M_{age}	Global	0.1	Aging mortality (per turn)
Sex	Individual	Male or female	The two sexes
Genotype	Individual	+/+ or +/t	Every individual is diploid, carrying two haplotypes with four loci that shape their dispersal propensity.
Age	Individual	0 at birth	Age increments with 1 each turn. From age 1 on, the mice can mate. Mice will disperse depending on their dispersal propensity only at age 1 exactly. After that, they remain in their general area.
$N_{\text{max-matings}}$	Individual	1 or 3	(Females only) The maximum number of a times a female will mate per turn. A female that is receptive to mating multiple times (dependent on P_{multi}), will mate up to three times.

Variable	Type	Value	Description
D_0	Locus	-3 to 3	First bound of dispersal propensity (i.e. highest or lowest migration propensity).
D_1	Locus	-3 to 3	Second bound of dispersal propensity (i.e. highest or lowest migration propensity).
D_2	Locus	0 to 5	Steepness of the change from the first to the second bound.
D_3	Locus	-300 to 300	Local density at which the change from first to second bound is at its midpoint.
K_p	Patch	1 to 10 (integers)	Each patch is assigned this variable randomly at initialization. This variable determines the carrying capacity (see *Mortality*).

3.3.8 Execution and analysis of the simulations

We ran 25 simulations for 10,000 turns each per condition. To visualize the evolved dispersal functions, we combined all simulations with the same condition, selected turns 9,990 to 10,000, randomly selected up to 50,000 individuals per genotype and computed each genotype's mean dispersal probability for local mouse densities (N_{R_3}) ranging between 0 and 80, which was the realized range in the simulations (see Appendix Figure 6.4). Since the parameter space allowed for reaction norm values to range between -3 and +3 (with all values below 0 phenotypically equaling no dispersal and above 1 phenotypically equaling certain dispersal), we restricted the values back to the phenotypic range

between 0 and 1 ("phenotypic dispersal propensity", see *Dispersal*) when comparing differences in the phenotypes between the genotypes.

3.4 Results

3.4.1 Verification of the simulation

The stochastic carrying capacity distribution throughout the simulated worlds led to an approximately normal distribution of mice in R_3 (the local density evaluated by the mice for density-dependent dispersal) with a mean of 26.79 and a standard deviation of 10.21 mice under natural conditions (see Appendix Figure 6.4), thus we succeeded in creating a heterogeneous environment. For our hypothesis it is important that there is a relationship between local density (which the mice evaluate with regards to their dispersal decision) and polyandry (to which the mice are blind). To verify that the simulated variation in local density generated variation in polyandry, we investigated the relationship between local density and the number of male partners that females had per turn and found that both the proportion of females receptive to mating multiply and the local density have a major impact on the proportion of realized matings that are polyandrous (Figure 3.4). When every female was assumed receptive to mating multiple times (which we consider the natural condition given that density predicts female multiple mating frequency in the wild (Dean et al. 2006; Firman and Simmons 2008)), we see considerable density-dependent variation in the proportion of realized matings that are polyandrous.

The frequency of +/*t* mice in the populations averaged 0.22 (SD=0.05) under the natural condition of our simulation (see Appendix Figure 6.5). This is in line with local +/*t* frequencies between 0.14 and 0.31 in nature (Ardlie and Silver 1998) (excluding populations where the *t* is very rare or absent), which suggests that the general dynamics of the *t* in our simulations are realistic.

3.4.2 The *t* evolves an increased density-dependent dispersal

Under natural conditions ($M_{\text{disp}} = 0.1$, $P_{\text{multi}} = 1.0$, and lethal *t* homozygotes), the *t* chromosome evolved a very distinct dispersal reaction norm (see the color

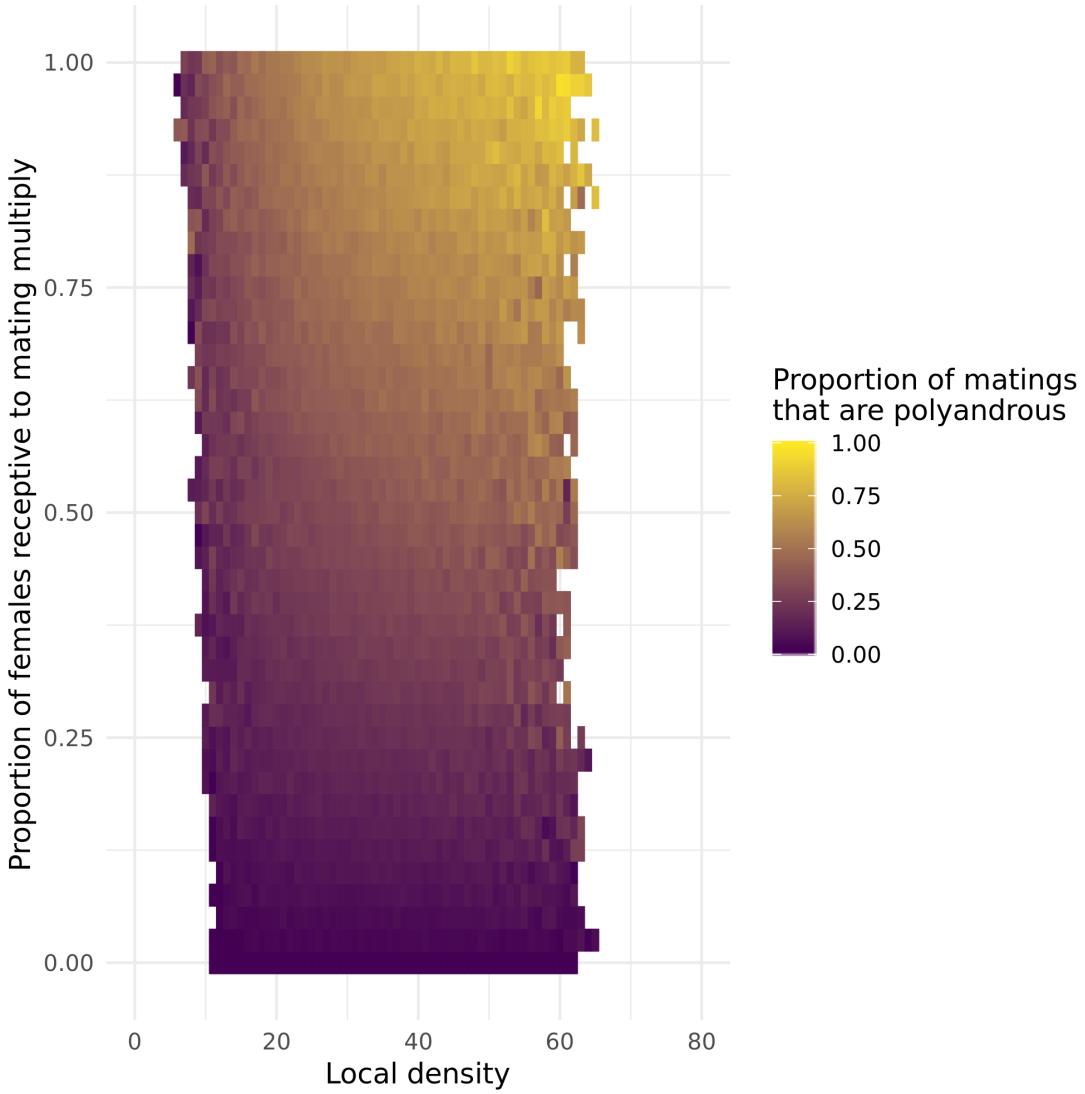


Figure 3.4: The relationship between proportion of females receptive to mating multiply (P_{multi}), the local density in radius R_3 around the focal females, and the proportion of realized polyandrous matings. The data is based on the final turn in simulations with dispersal cost $M_{\text{disp}} = 0.1$ and homozygous lethal *t* haplotype. Only data points with at least 25 observations are shown (mean = 637).

change from yellow to red in the highlighted part of Figure 3.5e and the red line in Figure 3.5g). Although *t* carriers rarely dispersed out of low densities, they still were more prone to do so than +/+. The difference was much more marked out of high density areas, with *t* carrier dispersal clearly exceeding that of the wildtype. The wildtype evolved nearly completely sedentary behavior, with almost no increase with the local density; the mean genotypic value for +/+ remained below 0.

3.4.3 *t*'s polyandrous disadvantage selects for positive density-dependent dispersal

We next modified the natural condition by varying dispersal mortality M_{disp}, the frequency of females receptive to mating multiple times per turn P_{multi}, and the effect of *t* homozygosity, to understand the consequent effects on dispersal propensity differences between +/*t* and +/+.

The increased dispersal of +/*t* occurs almost universally (see the amount of red and absence of black in Figures 3.5a-f), but it disappears in the combination of high M_{disp}, low P_{multi}, and semi-viable *t* homozygotes (see the mid to lower part of Figures 3.5f). All three conditions must be fulfilled, otherwise the difference between +/*t* and +/+ re-emerged. As a general pattern, semi-viable *t* homozygotes selected for a smaller increase in dispersal of +/*t* over +/+ (Figures 3.5d&f *vs.* 3.5c&e), with one clear exception: when dispersal had no costs ($M_{\text{disp}} = 0.0$), +/*t* dispersed a lot more than +/+ (particularly under high P_{multi}). However, this was mostly driven by +/+ dispersing less, possibly because under such extreme—and unnatural—circumstances, + only performs well at high densities where it can eliminate *t* through polyandry. When considering each genotype's dispersal propensity individually, +/*t* consistently dispersed more when homozygotes were lethal compared to when they were semi-viable (see Appendix Figures 6.6 and 6.8).

Even so, the density-dependent increase in dispersal propensity is mainly driven by M_{disp} and P_{multi}, not by homozygous costs. Costly dispersal (at least moderate M_{disp}) and high potential for polyandry are required for the dispersal difference between +/*t* and +/+ to show a substantial increase with density. Clearly density-dependent dispersal in +/*t* requires the frequency of females

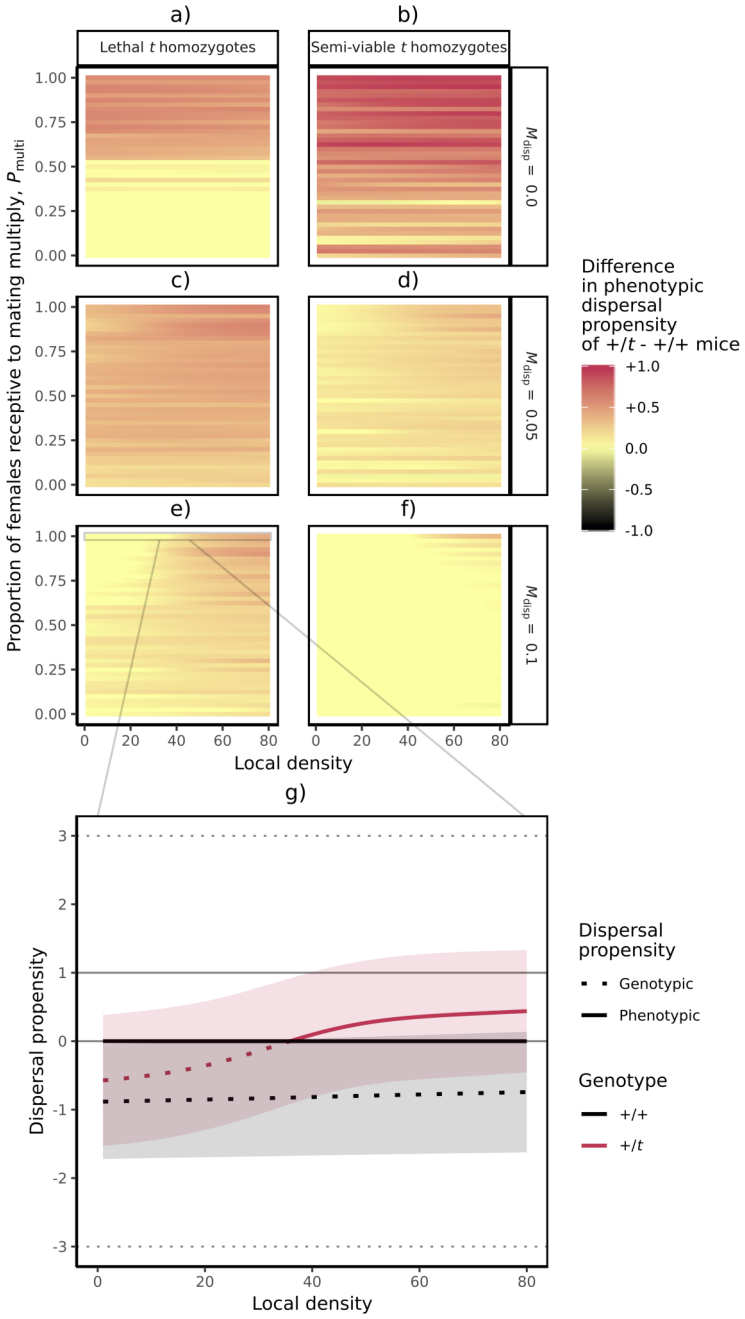
that were receptive to mating multiple times to be at least 75% (see the color change with density in the upper parts of Figures 3.5c-f), roughly corresponding to densities where polyandry begins to be more responsive to local density (see Figure 3.4, i.e. at that frequency of polyandry-receptive females, the local density started to matter for the *t*'s fitness). A weaker relationship between dispersal propensity and density can also be seen for lower P_{multi}, if M_{disp} is sufficiently high (Figure 3.5e), which selects against dispersal in those circumstances (low densities) where it is unlikely to help.

There was little difference in dispersal phenotypes when $M_{\text{disp}} = 0.0$, $P_{\text{multi}} < 0.25$, and *t* homozygotes were lethal (note the yellow lower part of Figure 3.5a); this was due to both genotypes evolving to always disperse across all densities (see Appendix Figures 6.6-6.7). There was also a lack of difference when $M_{\text{disp}} = 0.1$, $P_{\text{multi}} < 0.75$, and semi-viable *t* homozygotes (Figure 3.5f), but here it was caused by neither genotype dispersing (see Appendix Figures 6.6-6.7).

Figure 3.5: a-f) Overview of the mean difference in dispersal phenotypes between the genotypes. The phenotypic difference is taken from first restricting the evolved genotypic dispersal propensity to the effective phenotype (between 0.0 and 1.0), followed by subtracting the +/+ phenotype from the +/*t* phenotype. Yellow indicates no difference in phenotype, red indicates +/*t* as more dispersive, and black indicates +/+ as more dispersive. The proportion of females receptive to mating multiply, P_{multi}, is on the y axis, the local density is on the x axis, rows differ in dispersal cost M_{disp}, columns differ in *t* homozygous effect. g) Average evolved dispersal phenotypes in simulations with the natural condition ($M_{\text{disp}} = 0.1$, $P_{\text{multi}} = 1.0$, and lethal *t* homozygotes). +/*t* in red and +/+ in black. The dotted bold colored lines represent the mean genotypic propensity to disperse (between -3.0 and +3.0), the lighter area represents its standard deviation. The solid bold colored lines represent the corresponding phenotypic dispersal propensity (restricted to values between 0.0 and 1.0). The smaller grey solid lines indicate the effective phenotype range between 0 and 1 and the smaller dotted grey lines indicate the range of values that the genotypic dispersal propensity could take.

3.4.4 The wildtype adjusts to *t*

Above, we let both +/*t* and +/+ evolve in a situation of coexistence. To understand whether +/+'s dispersal is impacted by the behavior of +/*t*, we compared the evolved dispersal functions of +/+ in simulations with +/*t* to simulations in which there were no +/*t*. The phenotypic dispersal propensity of +/+ responded to the presence of +/*t* in simulations with lower M_{disp} (see SI for figures).
The effect of the presence of +/*t* on dispersal +/+ depended on P_{multi}. With no


a)
b)
Lethal t homozygotes
Semi-viable t homozygotes
c)
d)
e)
f)
g)
Proportion of females receptive to mating multiply, P_multi
Local density
M_disp = 0.0
M_disp = 0.05
M_disp = 0.1
Difference in phenotypic dispersal propensity of +/t - +/+ mice
+1.0
+0.5
0.0
-0.5
-1.0
Dispersal propensity
Dispersal propensity
Genotypic
Phenotypic
Genotype
+/+
+/t

costs to dispersal, *t*'s presence increased wildtype dispersal at low polyandry, but decreased it at high P_{multi}. In comparisons involving semi-viable *t* homozygotes, wildtype dispersal was now almost always decreased in the presence of *t*, the effect being stronger at high P_{multi}.
At moderately costly dispersal, the presence of *t* made +/+ disperse more when P_{multi} was low, while they dispersed a little less out of high densities when P_{multi} was medium to high. On their own, dispersal of +/+ required low M_{disp} (independent of any other variable), except for some dispersal out of high densities under medium M_{disp}. In summary, the results of this section yield two insights. First, modest costs are sufficient to reduce dispersal of +/+. Second, when dispersal costs are low enough and wildtypes coexist with *t*, dispersal as a whole is much more prevalent: +/*t* disperses more than +/+, but wildtype dispersal can be elevated too, especially when *t* spreads efficiently (low P_{multi}, combined with high fitness costs to *t* due to homozygosity (lethal *t* homozygotes)).
In addition to the phenotypic effects, + might also adapt more subtly to the presence of *t*. We next turn to differences in the genotypic dispersal propensity, i.e. not just the effective phenotypic range of 0 to 1 propensity (see SI for figures). Some of the range of genotypic values does not influence the phenotype (e.g. a shift from -2 to -2.5, both yielding zero dispersal), but with some chance of influencing the dispersal propensity of +/*t* because of co-dominant expression in the *t*-carrying heterozygotes. Across all M_{disp}, presence of *t* decreased the genotypic dispersal propensity of wildtypes in high P_{multi}, but increased or yielded no difference at low P_{multi} in the presence of +/*t*. At the same time, +/*t* co-evolution decreased +/+ dispersal more strongly in higher densities and increased it more strongly in lower densities. Since these patterns prevail up to high M_{disp}, where we did not observe a change in +/+ phenotypes, we suspect that this is evidence of a conflict between + and *t* within +/*t*: wildtype fitness increased with density, while *t* experiences the opposite (decreasing fitness with density). When + genes are expressed in +/+ and +/*t* indivduals, contexts which pull the dispersal phenotype in opposite directions, the mean + dispersal propensity never reached the lowest parameter space limit of -3 dispersal propensity, as this could have actually prevented +/*t* from dispersing (to the detriment of the fitness of the chromosome responsible for this effect). Indeed, +'s genotypic dispersal propensity was even lower in simula-

tions with semi-viable than in simulations with lethal *t* homozygotes (where + was relatively less fit), but it was still not -3 (see SI for figures).

3.5 Discussion

Our results show that the *t* haplotype evolves a more dispersive phenotype than the wildtype under natural conditions (i.e. with all females receptive to mating multiply, *t* homozygous lethality, and considerable dispersal mortality). This difference is most pronounced at high local densities, prompting highest emigration rates for +/*t* individuals. By comparing the natural condition of the *t* haplotype with hypothetical conditions in which we varied the frequency of polyandry-receptive females, the harmfulness of *t* homozygosity, and the cost of dispersal, we were able to tease apart the consequences of different features of *t*. This allows us to demonstrate that *t*'s disadvantage in polyandrous situations, combined with at least some dispersal costs, are primarily responsible for the density-dependent elevation in dispersal propensity. The homozygous lethality of the *t* haplotype also increased the dispersal propensity of its carriers, but not in a density-dependent fashion.

The wildtype coevolves in intruiguing ways in the presence of *t*, not only because of phenotypic effects of *t* on local densities (via emigration and immigration of competitors and mates), but also because wildtype chromosomes are expressed in +/*t* heterozygotes. Generally, wildtypes respond to *t*'s increased genotypic dispersal propensity by decreasing their own dispersal propensity, but typically this did not result in phenotypic change, as the +/+ phenotype was already largely non-dispersing in wildtype-only simulations. Genotypic values of + did not evolve to be so low that their expression in +/*t* individuals would have prevented them from dispersing. This reveals a potential genetic conflict over the dispersal phenotype, with being in *t*-carriers selecting + chromosomes to promote dispersal, and the opposite effect in +/+ individuals. Thus, intriguingly, the dispersal phenotype of +/*t* is not optimized solely in the interest of *t*, but also in the interest of +, though to a smaller degree than what would be expected if +/*t* followed Mendelian segregation that gave + their "fair" prospects to be transmitted to the next generation (for expectations of suppression of the

selfish genetic element's phenotype manipulation see Scott and West (2019)). As a whole, our results help explain why +/*t* were found to disperse more than +/+ in a long-term study on free-living wild house mice (see Figure 3.1) (Runge and Lindholm 2018).

The disadvantage that *t* experiences in female multiple mating contexts has a pronounced effect on dispersal. It shapes +/*t*'s dispersal propensity into a density-dependent function, assuming that dispersal is costly. This is because the likelihood of females mating with multiple males increases with local population density (see Figure 3.4), a pattern also found in studies in the field (Dean et al. 2006; Firman and Simmons 2008). Therefore, high risks of dispersal are outweighed by the potential benefits for the *t* when dispersal allows emigration from a locally dense population, assuming conditions where females are willing to mate multiply. As the disadvantage in sperm competition appears to be a general issue with male meiotic drivers (Haig and Bergstrom 1995; Price et al. 2008), this result could be of interest in other systems as well.

The homozygous lethality of the *t* haplotype is likely to amplify the effect of kin selection, which further increases the dispersal propensity. Kin selection on its own selects for dispersal, because emigrating kin decrease competition experienced by relatives who stay behind. With lethal *t*/*t*, emigration also helps relatives avoid matings that potentially lead to offspring deaths (somewhat analogous to dispersal to avoid inbreeding, (Gandon 1999)). We see this effect in our model, where *t*'s homozygous lethality further increases the selective pressure to disperse for *t* to avoid matings between two carriers. Consequently, more costly homozygosity selects for +/*t* to leave high *t* frequency areas. We assumed no ability of individuals to detect *t* directly. In the absence of multiple mating, therefore, homozygosity selects for increased dispersal in general (density-independent, see SI for figure). With multiple mating, those areas with high *t* frequency are primarily the low density areas (because of locally low multiple mating frequencies), and now costly homozygosity selects for increased dispersal primarily out of low density areas. This is at odds with the selection induced by female multiple mating (dispersal primarily out of dense areas), yielding a middle ground (some dispersal out of low density areas, but much more out of high density ones, dependent on dispersal mortality) for the overall pattern.

Previous models of the *t* haplotype's at that time unknown dispersal pheno-

type predicted implicitly that there should be an increase in dispersal, either because wildtype-fixed populations should be easily infected by *t* (Lewontin and Dunn 1960), or because sub-populations carrying the *t* would go extinct frequently (Lewontin 1962), or because a reduction in +/*t* dispersers due to selection between sub-populations would lead to low *t* frequencies (Nunney and Baker 1993). These studies, however, could not take into account the *t*'s disadvantage in polyandrous contexts because it had not yet been discovered. Ours is the first model that looks at the effects of both deleterious traits on the dispersal phenotype.

We did not include potentially sex-specific dispersal phenotypes in our model; for example, one could speculate whether only +/*t* males should disperse at higher rates because of the problems their sperm encounter in multiple mating contexts. We chose this simplifying assumption primarily because we did not see an effect like this in the long-term field study (Runge and Lindholm 2018). Our results show that a potentially more easily evolvable, sex-independent effect could evolve. It is also conceivable that females, as mothers of some +/*t* sons, could also profit from moving to places where the *t* haplotypes tend to do well. To that end, a study that asked very different questions from ours has found that fitness benefits of dispersal that are reaped a few generations after a dispersing ancestor can still select for dispersal (Travis et al. 2009). Either way, selection towards increased dispersal of +/*t* females is likely weaker than on +/*t* males; despite this, we found clear differences in dispersal phenotype between +/+ and +/*t* when dispersal effects were constrained to be identical for both sexes.

There is an ongoing effort to create a male-determining-gene-carrying *t* haplotype drive system (*t-SRY*) to eradicate harmful house mouse populations (Piaggio et al. 2017; Kanavy and Serr 2017; Gemmell and Tompkins 2017). It is crucial for the safety and success of this project to understand the dynamics of the *t* in the wild (Manser et al. 2019). In this study, we have provided evidence that *t*-carrying mice can be expected to have an increased dispersal propensity, which could help them spread a modified *t* haplotype further than planned. It is therefore important to model the influence of increased dispersal when considering the impact of the *t-SRY* system in the wild.

Our study provides, to our knowledge, the novel result and explanation of how

an intragenomic conflict involving a meiotic driver can select for differences in dispersal of driver-carrying individuals. Changes in behavior of driver-carriers have so far rarely been documented. A comparable phenomenon is found in fire ants where colonies of ants carrying a driving supergene are differently organized than those of non-carriers (Wang et al. 2013; Ross and Shoemaker 2018). Another example comes from the increased mating rate of *Wolbachia*-infected flies (Champion de Crespigny et al. 2006). More commonly known, but yet with few cases described, are behavioral adaptations in those that do not carry the driver, usually with regards to mating behavior to avoid transmission of the driver to offspring (Wilkinson et al. 1998; Wedell 2013). In summary, we showed how drivers can evolve an increased dispersal of their carriers. With this, we add another layer to the already complex intragenomic conflict between the driver and the rest of the genome.

3.5.1 Acknowledgements

We thank Andrés Bendesky and Barbara König for computing resources during parts of this study. This study was funded by the Swiss National Science Foundation (31003A_160328).

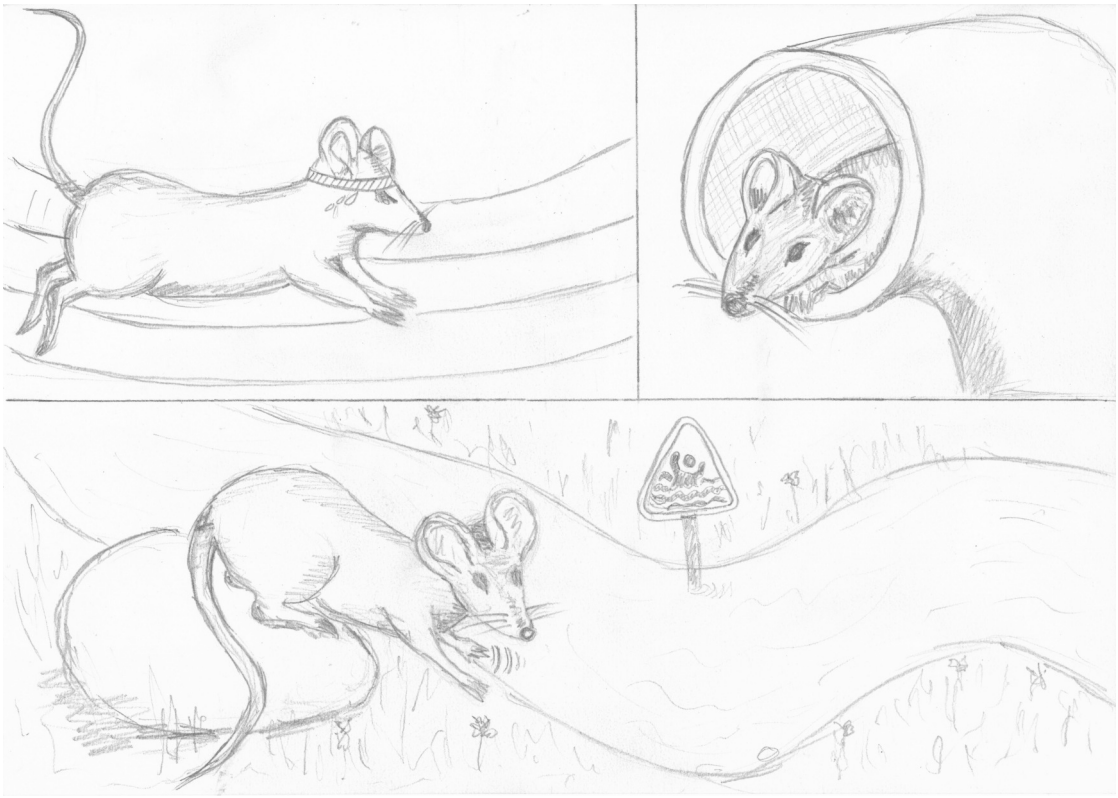
3.5.2 Author contributions

JNR conceived the study, programmed the simulation, and analyzed the data. JNR and HK contributed to simulation design. JNR, HK, and AKL wrote the manuscript.

3.5.3 Data availability

The code of the simulation will be available at https://github.com/jnrunge/t-vs-w-dispersal-evolution/ when the manuscript is published.

4 Experiments confirm a dispersive phenotype of house mice carrying a gene drive system



Germaine Léa Bongenge

Jan-Niklas Runge & Anna K. Lindholm

4.1 Abstract

Meiotic drivers are a type of genetic entity that increases its own probability of being transmitted to offspring to the detriment of the rest of the organism, thus "selfishly" increasing its fitness. This induces selection on the rest of the genome to suppress the increased transmission of the driver. In many meiotic drive systems, driver-carrying males are less successful in sperm competition, which occurs when females mate with multiple males in one oestrus cycle, and increased female remating rates can evolve to suppress the driver. In dense populations, where sperm competition is more common, drivers can even go extinct. How do drivers respond to this selection? House mice carrying the *t* haplotype, a meiotic driver, have been found to be more likely to disperse in a long-term field study. Evolutionary models also predict that the *t* haplotype should increase the dispersal propensity of its carriers because of the large fitness gains that *t* can accrue in ideal target populations. However, as of yet, no controlled experiments have been conducted to test these findings. Under experimental conditions, we found that carriers of the *t* haplotype were more dispersive. Heavier (better condition) *t*-carriers were particularly more likely to disperse than wildtype mice. Carriers of the *t* haplotype were also more explorative but not more active than wildtype mice. These findings shape the picture that the *t* haplotype produces a dispersal polymorphism in house mice, which is almost unique in mammals, and so far never discovered before in the world of meiotic drivers.

4.2 Introduction

Not all elements that make up the genome cooperate to increase the fitness of each other and of the individual that they produce (Burt and Trivers 2006). For example, meiotic drivers manipulate the products of meiosis, usually sperm, to increase their own chance of transmission to the next generation, thereby decreasing the fitness of competing elements on the homologous chromosome (Lindholm et al. 2016). Indeed, they often also decrease the fitness of the individual, thus only increasing their own. Hence, such elements are also called selfish genetic elements. As a consequence of their "selfishness", selfish ge-

netic elements induce selection on the rest of the genome to suppress their activity. The typical suppression takes place on the level of the genome, either by the chromosome most negatively affected by the selfish genetic elements or from elsewhere in the genome (Jaenike 1999). But suppression can also arise behaviorally, on the level of the individual, for example via mate choice favoring mates that suppress the selfish genetic elements (Wilkinson et al. 1998) or by exploiting sperm-competitive weaknesses of carriers of selfish genetic elements in polyandrous matings (matings in which females mate with multiple males) (Price et al. 2008). How does the selfish genetic element respond to the selection imposed by such suppression?

We investigate this question using a selfish genetic element found in house mice *Mus musculus*, the *t* haplotype. The *t* haplotype is a variant of the proximal 35 Mb of one of the house mouse autosomes (Kelemen and Vicoso 2018). It is a supergene (Schwander et al. 2014) consisting of at least four major inversions, which reduces the recombination rate to almost zero, thereby usually transmitting the *t* haplotype unchanged to the next generation (Kelemen and Vicoso 2018). Male heterozygous carriers of the *t* haplotype (notation: male +/*t*) transmit the *t* haplotype to over 90% of their offspring (instead of 50%), because *t* manipulates spermatogenesis in its favor (Burt and Trivers 2006; Lindholm et al. 2019), making it a meiotic driver. However, the *t* haplotype has major fitness drawbacks (Dunn and Levene 1961; Carroll et al. 2004; Safronova 2009; Manser et al. 2011, 2017; Sutter and Lindholm 2015). Mice homozygous for the *t* are not viable (Klein et al. 1984) or sterile as males (Lyon 1986) and male +/*t* perform very poorly in sperm competition, siring almost no offspring in female multiple matings (Manser et al. 2011, 2017; Sutter and Lindholm 2015). Recently, we found that juvenile carriers of the *t* haplotype were more likely to emigrate—the first step of dispersal (Matthysen 2012)—from a long-term study on free-living house mice, particularly in higher population densities (Runge and Lindholm 2018). We demonstrated that it is likely the disadvantage in female multiple mating—which is more frequent in higher densities (Manser 2015)—that drives the evolution of this trait using individual-based models (Chapter 3). Controlled experiments are however necessary to provide robust evidence for an increased dispersal tendency associated with the *t* haplotype. If independent observational, theoretical, and experimental evidence agree, then this observed

increased dispersal tendency associated with the *t* supergene is unlikely to be a false positive result.

An increased motivation to disperse could be associated with a suite of other behavioral differences as part of a "dispersal syndrome" (Matthysen 2012; Ronce and Clobert 2012). Dispersal syndromes describe the values of traits that are found exclusively or predominantly in dispersing individuals. For instance, winged morphs are the predominant dispersers in locusts (Williams 1957), and the *Pgi* polymorphism in butterflies is associated with greater dispersal, higher mobility, and increased lifespan (Niitepõld et al. 2009; Saastamoinen et al. 2009). In mammals, dispersal polymorphisms are only known from mole rats, which have an infrequent dispersal morph with increased body weight, changed hormone levels, high motivation to disperse, and increased interest in matings outside of their colony (O'Riain et al. 1996; Ronce and Clobert 2012). How individuals explore is often linked to dispersal in empirical studies, with dispersers being usually, but not always, more exploratory (Clobert et al. 2009; Cote et al. 2010). For example, exploration behavior positively predicted dispersal in roe deer (Debeffe et al. 2013) and natal dispersal was positively linked to exploration speed in great tits (Dingemanse et al. 2003). Another behavior that was found to be associated with dispersal is locomotor activity (Cote et al. 2010), for example in the dispersal morph of naked mole-rats (O'Riain et al. 1996) or in fruit flies selected for increased dispersal (Tung et al. 2018), who also showed more exploratory behavior. The links between dispersal and exploration and/or activity that have been found support the idea that a genotype that increases dispersal propensity could also modify activity or exploratory behavior to improve odds of successful dispersal.

Alternatively, dispersal could be mostly induced by environmental and internal cues (Lidicker and Stenseth 1992; Matthysen 2012), such as density (Matthysen 2005), condition (Ims and Hjermann 2001), and availability of defensible territory (Moore and Ali 1984), with motivation independent of these cues usually taking a minor role, with +/*t* perhaps being an exception due to stronger selection towards a general predisposition to disperse (Chapter 3).

Here we test whether heterozygous +/*t* mice differ from wildtype +/+ mice in their dispersal, activity, and exploration phenotypes using replicated experimental setups, with a design adapted from König et al. (2015) and Krackow

(2003). Dispersal was tested in enclosure setups with different densities of mice. We hypothesized that 1) +/*t* would disperse more out of dense enclosures than +/+ in line with previous findings, 2) +/*t* would be more exploratory than +/+, and 3) +/*t* would be more active. Secondly, we investigate whether these phenotypes are correlated with one another to better understand what traits are associated with the *t*, and why.

4.3 Methods

4.3.1 Study animals

The house mice *Mus musculus domesticus* used in this study were all lab-bred descendants from mice caught in an intensively studied free-living population of wild house mice (König et al. 2015). Therefore, these mice are genetically wild mice. After weaning at 23 days of age, the mice were on average 37.5 days of age, at least 26 and at most 49 days, when they were taken from same-sex sibling cages from the breeding lab, weighed, and placed separately into Macrolon Type II cages (267x207x140mm) with *ad libitum* access to food (laboratory animal diet for mice, Provimi Kliba SA, Kaiseraugst, Switzerland) and water. The cages were outfitted with bedding (Lignocel Hygienic Animal Bedding, JRS, Rosenberg, Germany), kitchen paper, a card board toilet paper roll, and cardboard pieces to provide hiding and nesting opportunities. Mice were kept alone for 7 to 13 days before the enclosure experiment began. This variation is due to some cohorts not taking part in the exploration experiment, plus/minus a day for organizational reasons, due to the time it takes to set up the enclosures. However, all mice of one enclosure cohort spent the same number of days in solo cages before entering the enclosures, with the exception of one mouse that replaced a mouse that died suddenly before the beginning of the enclosure experiment. During this time period, we conducted the activity and then the exploration experiments. After the activity and exploration experiments, the mice were injected with a transponder tag so that we could identify them reliably using handheld readers.
Carriers of the *t* haplotype were determined by amplification of the *Hba-ps4* locus (Schimenti et al. 1990) from ear punches taken from 13 day old pups, as in

Lindholm et al. (2013). +/+ mice were derived either from +/+ × +/+ crossings or from male +/+ × female +/*t* crossings. +/*t* mice were derived only from the latter. The male +/+ × female +/*t* crossings ensured that there would be an equal representation of +/*t* and +/+ in the offspring (the *t* does not drive in females) and no reduced litter size, like one would find in +/*t* × +/*t* crossings (Lindholm et al. 2013). A cohort of mice was selected to be 50% +/*t* and also 50% female, equally distributed between the genotypes, while matched in ages as close as possible. In the low density treatment, all mice came from different breeding pairs, while in the high density condition eight breeding pairs contributed one male and one female each to the enclosure. Age-matching was prioritized within sexes, so as to avoid competitive advantages. In the end, the genotypes and sexes did not differ in their age (adjusted R^2 = 0.004 of a linear model of age with genotype and sex as predictors).

4.3.2 Experimental setups

All experiments were conducted blind with regards to the genotype of the mice in so far as only individual IDs were immediately accessible at any point of the experiments and genotypes were not noted down in the locations where the experiments were conducted. Genotype at the *t* haplotype is not associated with any visible external phenotype.

4.3.3 Analysis

All plotting and analyses were perfomed in R 3.6.1 using the packages boot 1.3-23 (Davison and Hinkley 1997; Canty and Ripley 2019), corrplot 0.84 (Wei and Simko 2017), dplyr 0.8.3 (Wickham et al. 2019), ggplot2 3.2.1 (Wickham 2009), lme4 1.1-21 (Bates et al. 2015), MASS 7.3-51.5 (Venables and Ripley 2002), pbkrtest 0.4-7 (Halekoh and Højsgaard 2014), readr 1.3.1 (Halekoh and Højsgaard 2014), readxl 1.3.1 (Wickham and Bryan 2019), readODS 1.6.7 (Schutten et al. 2018), reshape2 1.4.3 (Wickham 2007), sjPlot 2.8.1 (Lüdecke 2016).

4.3.4 Testing dispersal

Twelve groups of either eight (low density, $n = 7$) or sixteen (high density, $n = 5$) mice were placed into seven square meter outdoor enclosures, for a total of 136 mice (25% each sex/genotype combination). The enclosures were designed to resemble the environment in which mice from the long-term study analyzed in Runge and Lindholm (2018) lived, but on a smaller scale. This experiment had two purposes: to test for dispersal differences, and to follow gene frequencies across generations (not reported here). Therefore mice were allowed to reproduce freely, for on average 107 $\pm$ 28 (SD) days before enclosure experiments were terminated. These experiments were conducted from April 2017 to December 2017 and June 2018 to November 2018.

The enclosures consisted of concrete floors, upon which we placed bedding material, the same we used in the cages, and walls to prevent mice from climbing out. The enclosures were themselves caged to protect against predation, and protected from rain with a plexiglass roof and from sun by a tarp hanging overhead. Each enclosure had four nest boxes, one in each corner, with one entry tube, representing high-quality nesting sites, and four low dividing walls arranged like a plus-sign with space in the middle of the enclosure and on the ends to allow for movements while also facilitating territory defense. We used tubes, bricks, sticks, tiles, stones, kitchen paper, straw, and cardboard rolls to provide hiding and evasion opportunities, nesting material, and enrichment. We had four feeding sites to which the mice had *ad libitum* access, filled with a half-and-half mix of hamster food (VITA-BALANCE 26267 by Landi AG, Switzerland) and oats, the same as used in König et al. (2015), including in the emigration study of Runge and Lindholm (2018). We also had four drinking sites per enclosure.

We created a dispersal opportunity (Figure 4.1) based on previous studies that successfully used water as a barrier for mice that, when crossed, was called a dispersal event (Gerlach 1996; Krackow 2003). Mice in the enclosures were able to leave via a tube that led out of the enclosure and towards an enclosed plastic box (290x200x220cm) filled with about 8cm deep water (called the “water cage”) with a tube on the other side leading to a Macrolon Type III cage (called the “dispersal cage”) with bedding, cardboard, food, and water, modeled after what

is used in our breeding lab and intended to be attractive. Both tubes leading in and out of the water were placed circa 4.5cm above the water with ladders (steel sheets with holes) covering the distance between tube and water. There was a divider from the top down to circa 11cm above the ground in the middle of each water cage to prevent the mice from jumping over the water. The access to this dispersal opportunity was ensured in the first two weeks of an experiment (excluding the weekend and/or short breaks for cleaning of the apparatus). Afterwards, access was regular but not constant, because mice started nesting in the tube leading to the water, which presumably would have interfered with dispersal attempts. Removing constant access was intended to discourage this behavior and whenever nesting occurred, the dispersal cage access was blocked off and the apparatus cleaned. Furthermore, 67% of dispersal took place in the first two weeks and 83% in the first three weeks, similarly to Krackow (2003) where most dispersal took place within 7 days. Thus, we assume that we found a good balance between continuous access to the water cage in terms of time and making sure that the water cage was actually accessible for all mice by having it clean and without nests, i.e. outside mice's territories. Each morning on weekdays (with access to the apparatus being blocked on weekends), the dispersal cage was checked for the presence of mice. Upon finding a mouse, the cage was removed with the mouse inside and the mouse was declared a disperser (and would not return to the enclosure). The dispersal apparatus was subsequently cleaned with soap and water and setup again.
Mice that founded the enclosures as well as mice that were born in the enclosures were able to disperse. Births in the enclosures were monitored with regular searches for new offspring, every 10 days. These offspring were tissue sampled for genetic analysis at 13 days of age and returned to the nests. To our surprise, we only ever recorded 2 out of over 500 offspring dispersing from the analyzed enclosures. Hence, we did not investigate that data further for this purpose and instead focused on the founders who dispersed much more often. Mice were able to return to the enclosure after crossing the water barrier. We decided against preventing the mice from leaving the dispersal cage once they entered it, because it would make it harder to interpret whether they actually wanted to leave the enclosure if they had no way of returning. We used video cameras to monitor a portion of the dispersal cages and, except for a few occa-

Figure 4.1: Photo of a dispersal experimental setup and about a third of one of the enclosures. The tube leads from the enclosure towards the water cage and from there to an intermediary cage that is connected to the high-value dispersal cage with food, water, and enrichment.

sions, mice did not move back to the enclosure.

4.3.4.1 Statistical analysis

The longest observed delay until dispersal was 53 days. As eight mice died prior to 53 days from the start of the trial (3 +/*t* males, 4 +/+ males, 1 +/+ female), we excluded them from the analysis, because we do not know whether they would have dispersed. We further removed one +/*t* female because we could not reliably determine her fate, due to the poor quality of her DNA sample and the loss of her transponder.

We built binomial generalized linear models with dispersal (1 or 0) as the response variable and treatment (high or low density), age at start, weight at start,

and sex as controlling variables. We then tested the effect of genotype by comparing models with and without this effect using model comparisons based on parametric bootstrapping with 10,000 replications, of which we then evaluated percentage of replicates that had a larger likelihood ratio test statistic than the one we observed, which served as the p-value, using the R package pbkrtest. We also tested the hypothesized interaction between genotype and treatment in the same way. Finally, all other possible interactions with controlling variables were also tested. We then described each predictor's effect with bootstrapped 95% confidence intervals using the function confint and the method "boot", with the type "basic". For this computation, we used a model with genotype and the controlling variables when describing the effect of the genotype and a model with all significant and hypothesized effects for other effect descriptions.
Similarly, we built negative-binomial distributed generalized linear models with dispersal delay (in days) as the response variable, but only including the dispersers ($n = 24$). Due to the small sample size, we only used the genotype as predictor and bootstrapped 10,000 times to get a confidence interval of the effect, which we would regard as significantly different from 0 if not overlapping 0.

4.3.5 Activity test

First, before entering the enclosures and the associated dispersal test, mice were given running wheels as a measure of locomotor activity. The data of mice, bred under identical conditions and selected using the same protocol, who did not enter the dispersal experiment, were added to these analyses. In total, 189 mice (25% each sex/genotype combination) entered the wheel running experiment.
One running wheel by Linton Instrumentation Ltd was fitted into the solo cage of each mouse and remained there for 72 consecutive hours. The activity of the wheel (when and how often it was turned by the mouse) was tracked with Columbus Instruments Device Interface 1.5 (Columbus, Ohio, USA). Due to resource constraints, we first gave half a cohort—50% +/t and 50% female—the running wheel for 72 hours, then removed the running wheels, cleaned them with soap and water, dried them, and placed the wheels with the other half of the cohort

for 72 hours. The running wheel was checked regularly to see whether its movement was blocked by nesting material, and cleaned when this was the case.

4.3.5.1 Statistical analysis

We recorded the revolutions of each wheel in 30-minute-windows (i.e. how many revolutions there were in 30 minutes). We first investigated the data visually by averaging the three days of wheel running to per hour-of-day averages. We discovered a strong difference between active and inactive phases during the day. For later analyses, we only analyzed the active hours, which were deduced to be from 21:00 to 06:00 (Central European Summer Time) based on visual inspection of the revolutions per hour of day. The active hours corresponded to the day/night cycle in the lab (dawn from 06:00 to 07:30, dusk from 19:30 to 20:30).
We excluded 11 individuals that had fewer than 16 revolutions in total (during the night) to ensure that the mice understood how to use the wheel. Afterwards, the least active individual had 191 revolutions and the most active had 50,238 revolutions (during the night). We created a linear mixed model with the response variable revolutions per hour, the random effect intercepts hour of night (as a factor) and individual ID. We controlled for sex, age, and weight. We then tested the effect of genotype using parametric bootstrapping model comparisons and described effect sizes with bootstrapping as well (see the statistics section of the dispersal experiment). We also compared models with genotype interaction effects to models without.

4.3.6 Exploration

After the six days (2x72 hours) of the running wheel experiment, mice were placed into the exploration test, which was conducted during day time hours. The exploration experiment was added to this study after the first cohort went into the dispersal experiment and one high density cohort was not tested in the exploration setup due to time constraints. Finally, one mouse was tested using an incomplete setup and thus removed, and one mouse died before entering the enclosure and the replacement (matching sex, genotype, age, and breeding family) for this mouse could not be tested to avoid delays in the dis-

persal setup. Thus, 110 out of 136 mice (26 +/+ males, 28 +/+ females, 28 +/*t* males, 28 +/*t* females) entered the exploration test. For each iteration of the exploration experiment, a mouse was removed from its cage and placed into a container (590x390x420mm), normally used to hold mice when cleaning cages. Inside this container, the mouse was introduced to a new cardboard toilet roll, like the ones used in each cage for enrichment, without directly touching the mice. This was done to minimize anxiety (Gouveia and Hurst 2013). The cardboard roll was placed directly in front of the mouse, with the side of the roll facing away from the mouse blocked by crumbled up kitchen paper, until the mouse entered it. Upon entry, the side of the cardboard roll that the mouse entered from was also quickly blocked with a similar amount of crumbled kitchen paper. Then, the entire cardboard roll was enclosed by a sheet of kitchen paper that was modestly tightly wrapped and knotted. This was done with the paper itself, such that all materials (cardboard and kitchen paper) were easily breakable (e.g. by biting) and already known by the mice as these materials were used extensively in the breeding lab and the solo cages. Therefore, mice who were motivated to leave the cardboard roll should have been capable to do so.
Mice in the wrapped cardboard roll were placed in the exploration setup, which consisted of Macrolon Type II cage in the center, connected by two tubes to two Macrolon Type III (382x220x150mm) cages on either side (Figure 4.2). All cages were closed with plexiglas lids, with some space left on the sides for air flow, leaving the mice visually unobstructed. A video camera was placed on a tripod such that it was angled downwards filming the setup from above. Once the mouse within the cardboard roll was placed into the middle cage and the plexiglas was put on top of the middle cage, the experimenters left the room and the mouse was left alone for 25 minutes, after which the mouse was removed from the setup and placed back into its cage. The setup was then cleaned with soap and water and dried before the next mouse was placed into it.

4.3.6.1 Statistical analysis

The videos of the exploration experiments were analyzed using the software BORIS (Friard and Gamba 2016). The movements of the mouse between the five compartments of the setup, the middle cage, the left and right cages, and

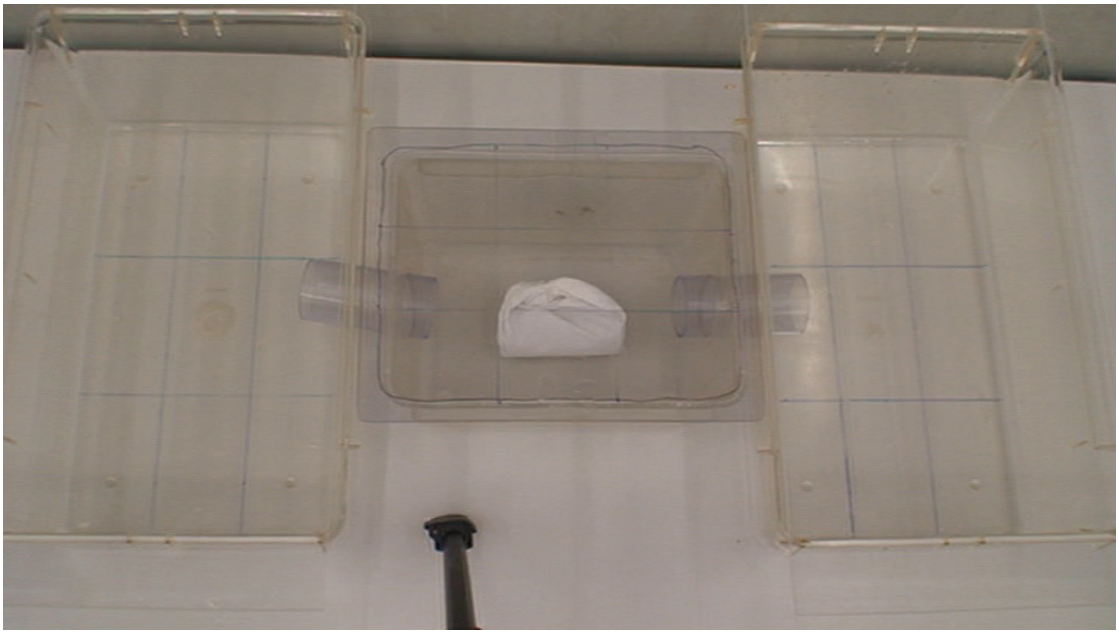


Figure 4.2: Still image of a video recorded as part of the exploration experiment. An enclosed cardboard roll with a mouse inside can be seen in the middle cage.

the two tubes connecting the middle cage to either side cage were scored by independent observers blind to sex and genotype. The time at which each of these compartments was entered was recorded (throughout the video, not just the first time it was entered). The first movement of a mouse would be into the middle cage, which was if and when the mouse left the cardboard roll.
For each mouse, we extracted seven measurements from the video analyses: how many unique compartments the mouse visited in total (0-5), how often the mouse would move from one compartment to another per minute after the mouse left the cardboard roll, and when it visited its first to fifth compartment for the first time. We combined these measurements into a PCA to avoid multiple testing with these highly correlated variables. To do so, we set the times at which a mouse entered the xth compartment for mice that did not enter x compartments to 25 minutes (the maximum length of the videos analyzed per individual), e.g. a mouse that only explored 4 compartments, would be recorded as exploring the 5th compartment at minute 25. This was done to not have individuals with missing data. Similarly, mice that never left the cardboard roll for more than 60 seconds were recorded as having had 0 movements between com-

partments per minute outside of the cardboard roll. PC1 explained 85.74% of the variance in this dataset. It was strongly correlated with all measurements in the direction of increased exploration, meaning more compartments explored, more movements between compartments, and quicker exploration of compartments (mean absolute *r* of 0.94, lowest absolute *r* was 0.87). Hence, we analyzed PC1 as the response variable "exploratiVeness". We could not get a similarly clear association with PC2, which explained 7.5% of the variance, and have thus not analyzed it further.
We created linear models with PC1 as the response variable, controlling for sex, age, and weight. Because of the violated linear model assumptions due to the unique distribution of PC1, we decided to not conduct typical testing, but instead evaluated the 95% confidence intervals of the predictor coefficients generated by resampling the predictor values from 10,000 bootstrapped replicates. We considered an effect to be statistically significant when the 95% confidence interval did not overlap 0. We did this for the genotype effect in the controlled model, and for each genotype interaction in a model with only this interaction and the controlled variables.

4.3.7 Dispersal syndrome

To discover and describe behavioral correlations between the three phenotypes—activity, exploration, and dispersal—we constructed a Pearson correlation matrix based on individuals that fulfilled all criteria for each analysis laid out above ($n = 94$), first with only three measurements, mean wheel running, exploration PC1, and dispersal (represented as 0 and 1). Then, after not finding any strong correlations, we created an extended correlation matrix by sub-dividing exploration into four key measurements that are part of the exploration PCA, namely time until mouse started exploring, movements between compartments during exploration, the number of compartments that the mouse explored, and whether the mouse explored at all (0 or 1). The latter measure was only implicitly excluded in the exploration PCA.

4.4 Results

4.4.1 Dispersal

24 out of 127 analyzed founders dispersed from their enclosures. Of those 24, 16 were +/t and 8 +/+. This was distributed unevenly between the densities, with 62.5% of +/t dispersal occurring in high densities, compared to 37.5% of +/+ dispersal (Figure 4.3). When analyzing the models, we found that +/t were more likely to disperse than +/+ ($p = 0.03$), with +/t on average having $\geq$ 300% higher odds to disperse (odds ratio: 3.17 (1.15 to 9.55)). Although in Figure 4.3 density appears to have a different influence on dispersal depending on the genotype, we did not find the predicted interaction between the density of the enclosure and the genotype to be statistically significant ($p = 0.18$). However, the direction of this interaction did line up with our expectations (see Figure 4.4): +/+ had on average almost 74.3% decreased odds to disperse in high densities compared to high densities (odds ratio: 0.26 (0.04 to 1.40)), but +/t had on average 22.3% increased odds to disperse in high densities (odds ratio: 1.22 (0.14 to 11.87)). We kept this interaction in the model for the following tests and coefficient descriptions because we hypothesized it.

Furthermore, we found that an interaction between genotype and an individual's weight (weighed before entering the enclosure) predicted dispersal probability ($p = 0.008$). For +/+, every gram of weight corresponded to a decrease in odds to disperse by almost half (odds ratio: 0.52 (0.34 to 0.75)). In contrast, for +/t an increase in one gram of body weight corresponded to only a 13% decrease in odds to disperse (odds ratio: 0.87 (0.60-1.30)). Thus, at higher weights, the dispersal probabilities diverged quite strongly (see Figure 4.4). There was no significant interaction with sex ($p = 0.12$) or age ($p = 0.25$ while still controlling for weight). In both genotypes, females had 97% lower odds to disperse than males (odds ratio: 0.03 (0.004 to 0.16)) and age had no clear influence, on average increasing odds to disperse by 9% per day of age (odds ratio: 1.09 (0.97 to 1.23)). Additionally, +/t were on average 0.99 grams heavier (0.25 to 1.72) when controlling for age (0.19 gram per day (0.12 to 0.26)) and sex (females: -4.25 grams (-3.51 to -4.99)).

Finally, we analyzed the difference in dispersal delay between +/t and +/+. The

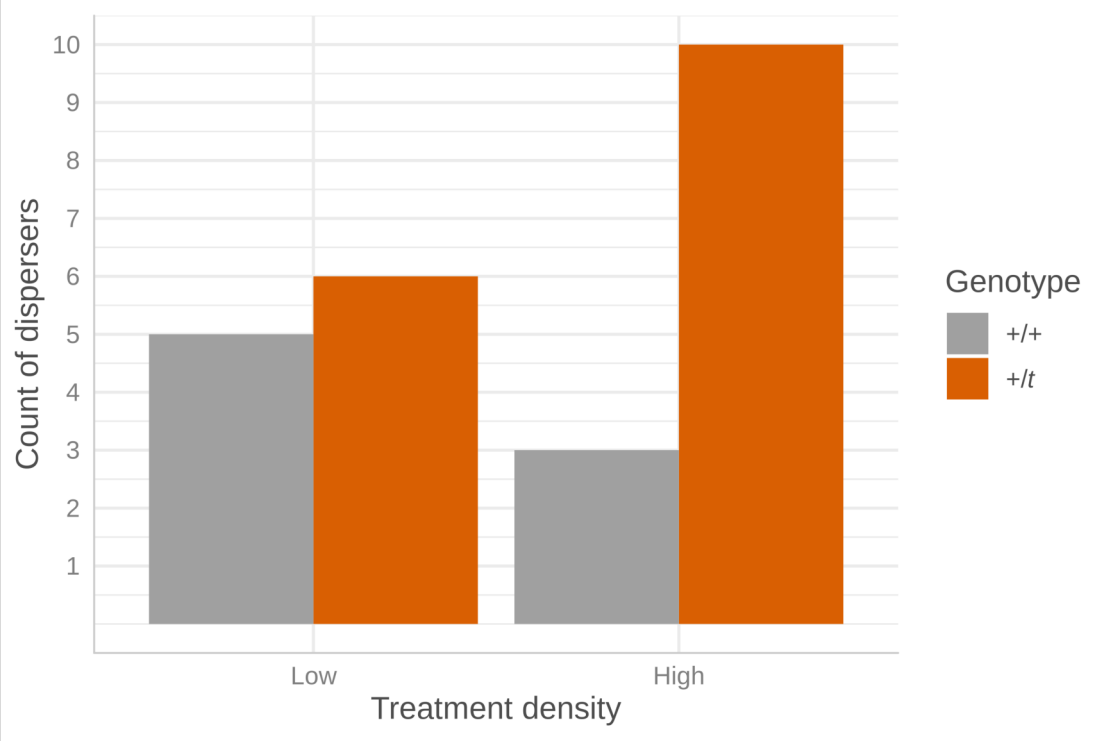


Figure 4.3: Raw counts of dispersers of the two genotypes in the two treatment densities ($n = 24$).

expected count of days until a disperser dispersed in a model of dispersal delay ($n = 24$) was on average 45.6% decreased in +/*t*, but the CI spanned from a 77% decrease to a 28.7% increase (mean difference in the logs of expected counts for +/*t* (β): -0.61 (-1.47 to 0.25). Therefore, the difference was not statistically significant, but trending in the direction of faster dispersal for +/*t* (see Figure 4.5).

4.4.2 Activity

+/*t* mice did not differ significantly from +/+ mice in their wheel running activity (Figure 4.6, $p = 0.67$, $n = 178$). Based on model predictions, males on average produced 323.03 (95% confidence interval (CI): -445.13 to -200.48) fewer wheel revolutions per hour than females. Age and weight did not have a significant influence ($p = 0.92$ and $p = 0.12$, respectively). Similarly, no interaction with genotype of any of the other variables (sex, age, and weight) was significant

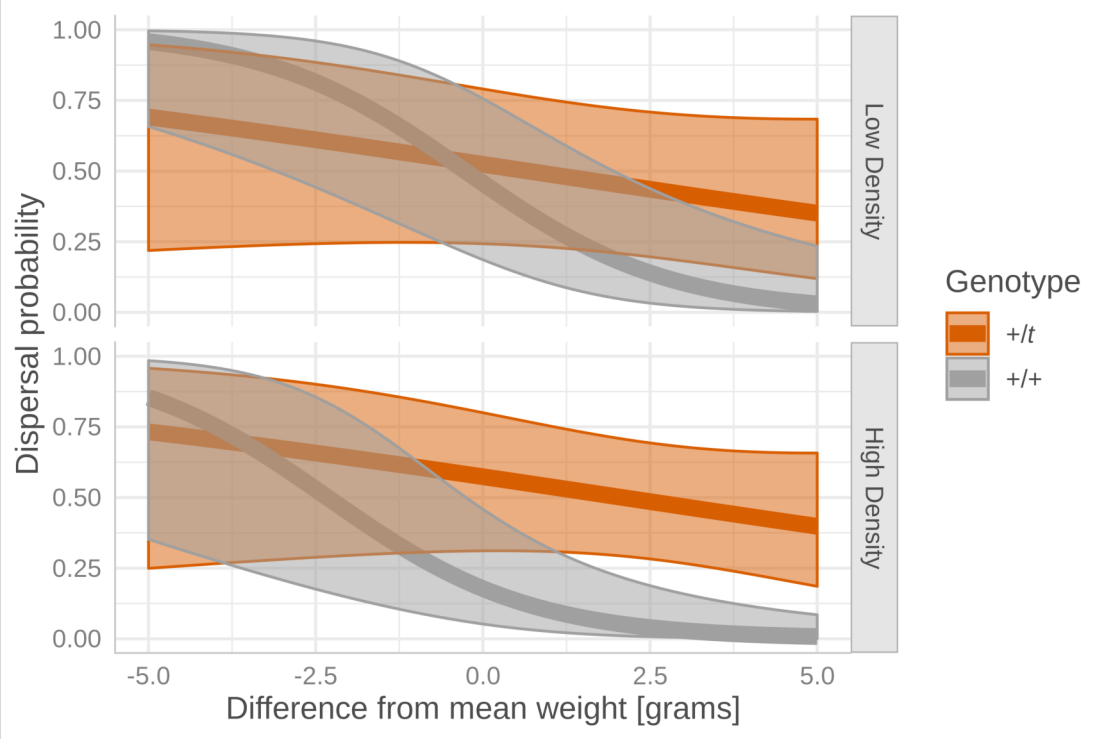


Figure 4.4: Predicted dispersal probabilities of generalized linear mixed models with interaction effects of genotype and weight as well as genotype and treatment, controlled for by age, weight, and sex. The shaded areas show the respective 95% confidence intervals for male +/+ and male +/*t* of different weights, separated into the two treatment densities.

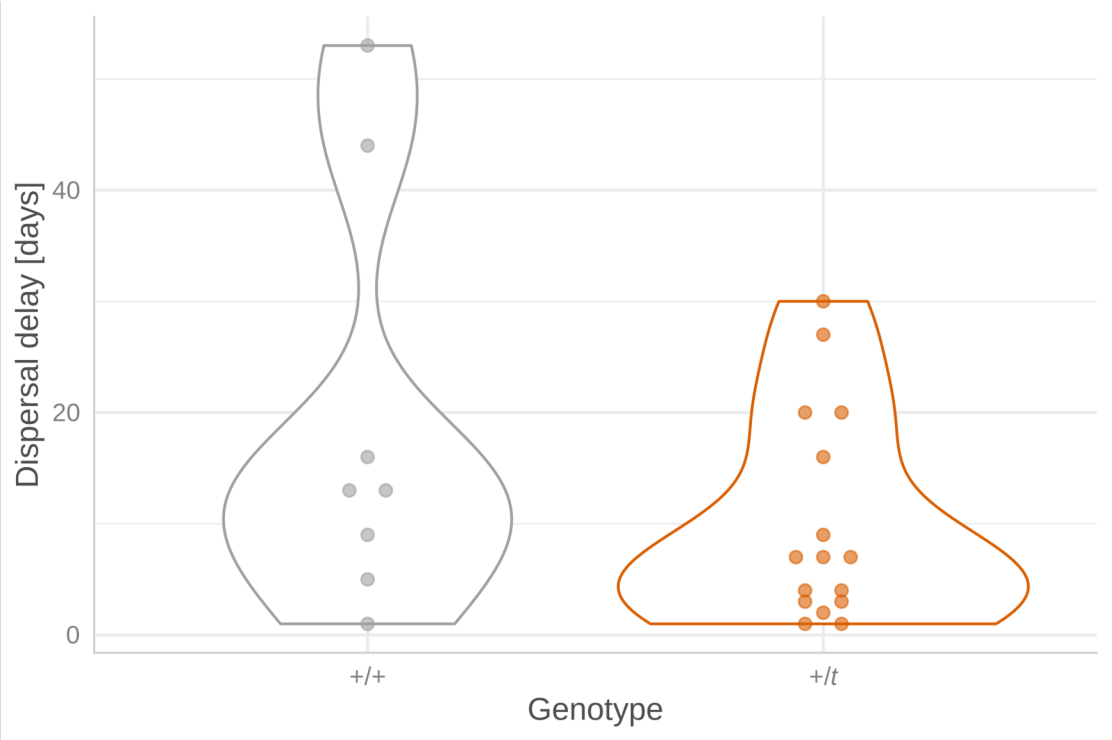


Figure 4.5: Violin plots including raw data points of the 24 dispersers and their individual delay in days until they dispersed.

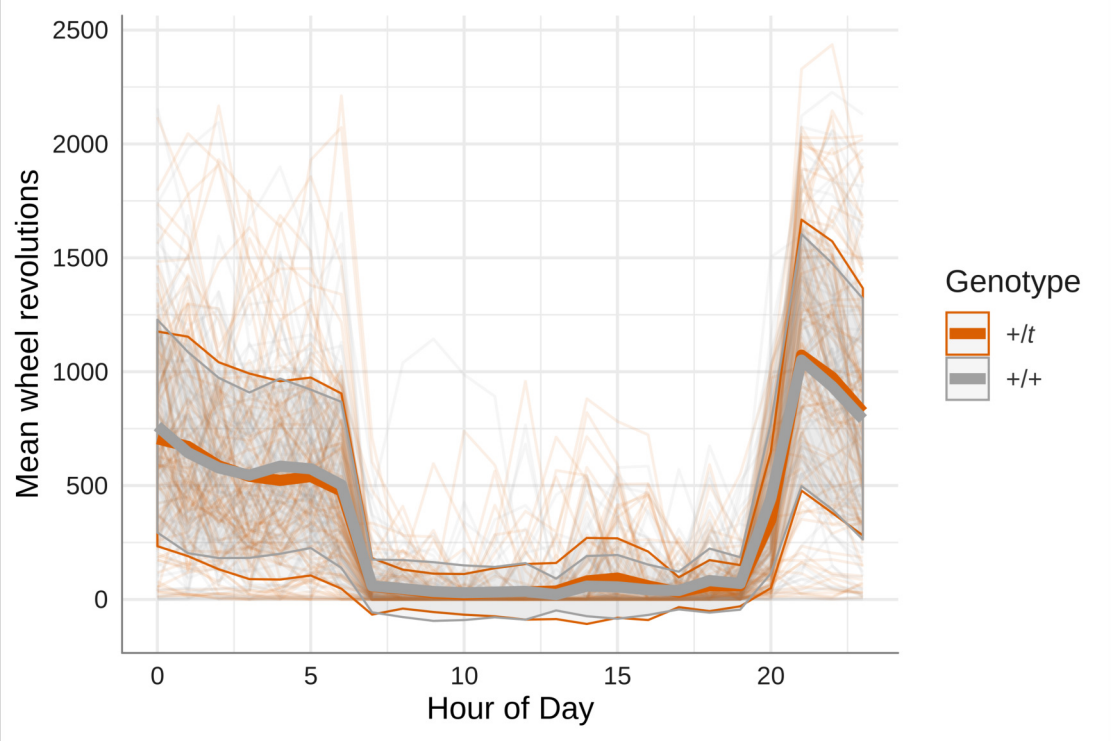


Figure 4.6: Average wheel revolutions (bold lines, standard deviation in shaded area) per genotype over the course of a day. Individual ($n = 178$) averages are plotted in translucent, smaller lines.

either ($p = 0.12$, $p = 0.21$, and $p = 0.87$, respectively).

4.4.3 Exploration

ExploratiVeness values (measured as PC1, thus with a mean approximating 0) ranged from -1.61 to +7.3 with 53.6% of the sample ($n = 110$) at -1.61, because all non-exploring mice had the same PC1 value (see Figure 4.7). Bootstrapped predictors of a linear model, controlling for age, sex, and weight showed that +/t had a PC1 that was increased (more explorative) by 1.05 (CI: 0.06 to 2.04). Since the CI did not overlap 0, we considered this effect to be significant. There was no significant interaction effect (Sex [males] interaction: -1.35 to 2.28; Age [days] interaction: -0.06 to 0.26; Weight [grams] interaction: -0.36 to 0.24). Sex, age, and weight did also not predict exploratiVeness in general, with age being the closest to passing our significance criteria (Sex [males]: -0.70 to 1.68; Age

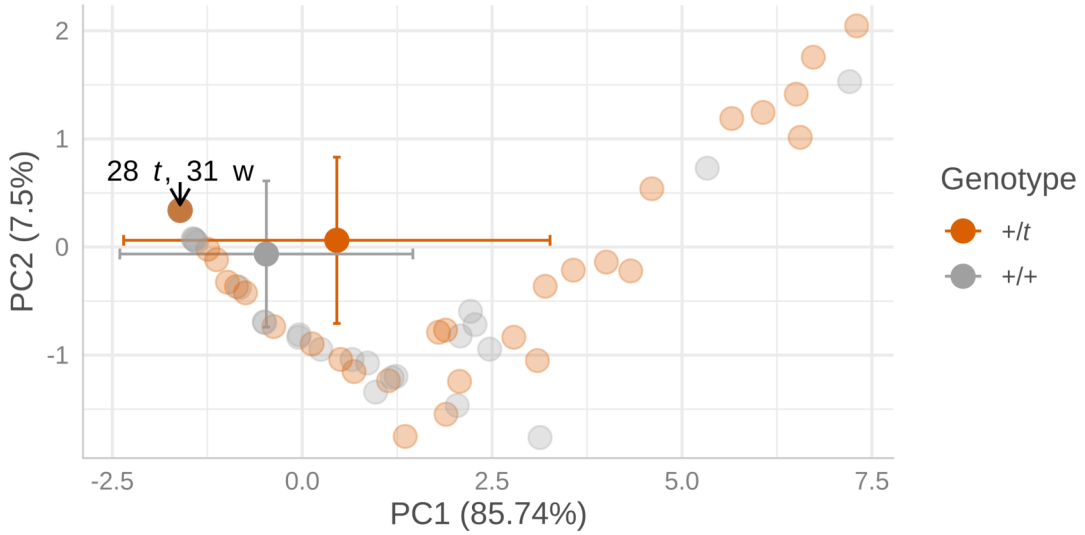


Figure 4.7: Individual ($n = 110$) PC1 and PC2 values of a PCA of exploration setup variables: how many unique compartments the mouse visited in total (0-5), how often the mouse would move from one compartment to another per minute since the mouse left the cardboard roll, and when it visited its first to fifth compartment for the first time. The dot with 59 non-explorers is highlighted with an arrow. The means of both genotypes are shown in non-translucent dots with the standard deviations as error bars.

[days]: -0.02 to 0.18; Weight [grams]: -0.23 to 0.21).

4.4.4 No clear evidence for a dispersal syndrome

Although +/*t* were more dispersive and more explorative, but not more active, it was not clear whether and how an individual's activity, exploration, and dispersal phenotypes are connected. We found only weak overall correlations between the three phenotypes (see Figure 4.8; $R = 0.19$ for exploratiVeness [PC1] ~ mean wheel revolutions, $R = 0.15$ for dispersal ~ exploratiVeness [PC1], and $R = -0.11$ for dispersal ~ mean wheel revolutions). Because *t* was associated with both dispersal and exploration, we decided to investigate an extended correlation matrix that included all observed variables of the exploration experiment that make up the PC1 that we termed "exploratiVeness". We found that the strongest correlation of an exploration variable with dispersal was found in whether the mice explored at all ($R = 0.25$), followed by variables most strongly correlated with this one (for example, how many compartments the

mice explored ($R = 0.23$)). However, all of these correlations were rather weak as well. Finally, we tested whether a combination of activity and exploratiVeness could predict dispersal, but an interaction between the two had no predictive power over a model without any predictors (binomial generalized linear model comparisons: $p = 0.27$). Whether the mice explored at all did predict dispersal positively ($p = 0.02$, model comparison), but was not in turn increased in +/*t* ($p = 0.31$, model comparison controlled for sex, age, and weight), therefore we could not establish a clear link between the two phenotypes more common in +/*t*. However, even the relationship between exploring at all and dispersal should be questioned given the amount of searching we needed to do to find it.

The strongest correlation between activity and an exploration variable was found in the movements between compartments ($R = 0.23$). This correlation was even stronger when only including mice that did explore at all ($R = 0.39$), implying that it was in fact the movements between compartments that were predicted by activity.

We also generated a similar correlation matrix with dispersal delay instead of dispersal (Figure 4.9), including only the dispersers who were tested for exploratiVeness ($n = 21$). Similarly to before, no strong correlations with dispersal delay emerged. The strongest one was once again whether the mice explored at all ($R = 0.27$). However, the correlation was positive, implying an increased dispersal delay in those mice that explored over those that did not. A similarly counter-intuitive correlation was found in number of compartments explored ($R = 0.27$). The next strongest correlation of dispersal delay was with wheel activity, here more wheel running activity correlating with earlier dispersal ($R = -0.19$).

4.5 Discussion

We confirmed that carriers of the *t* haplotype are more dispersive than mice that do not carry the *t* haplotype. Dispersal was tested using an established paradigm in which mice departing from a population by crossing a water barrier are defined as dispersers (Gerlach 1996; Krackow 2003). After a correla-

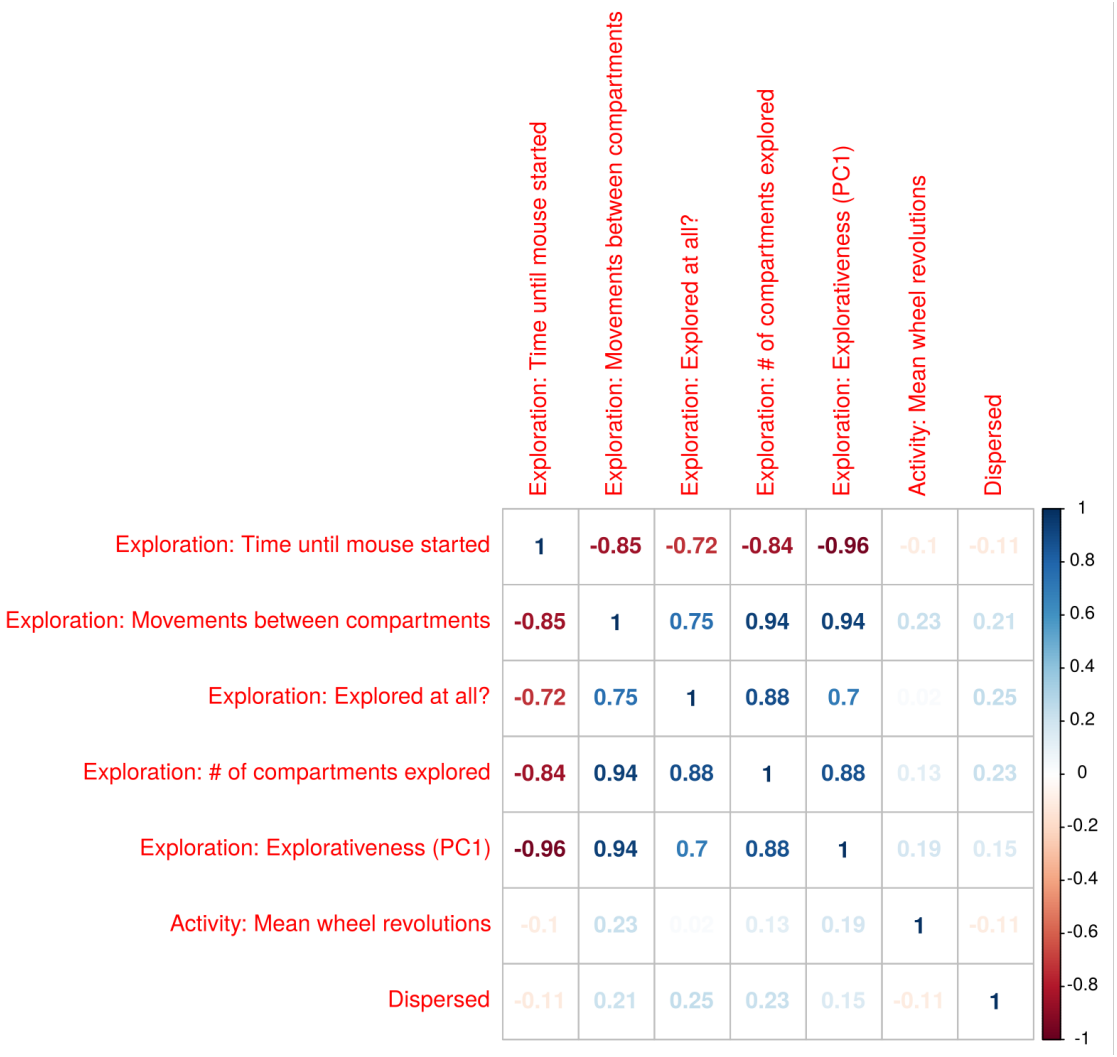


Figure 4.8: Pearson correlation matrix ($n = 94$) with four components of exploration and the combined measure explorativeness, activity measured in mean wheel revolutions, and dispersal (0 or 1).

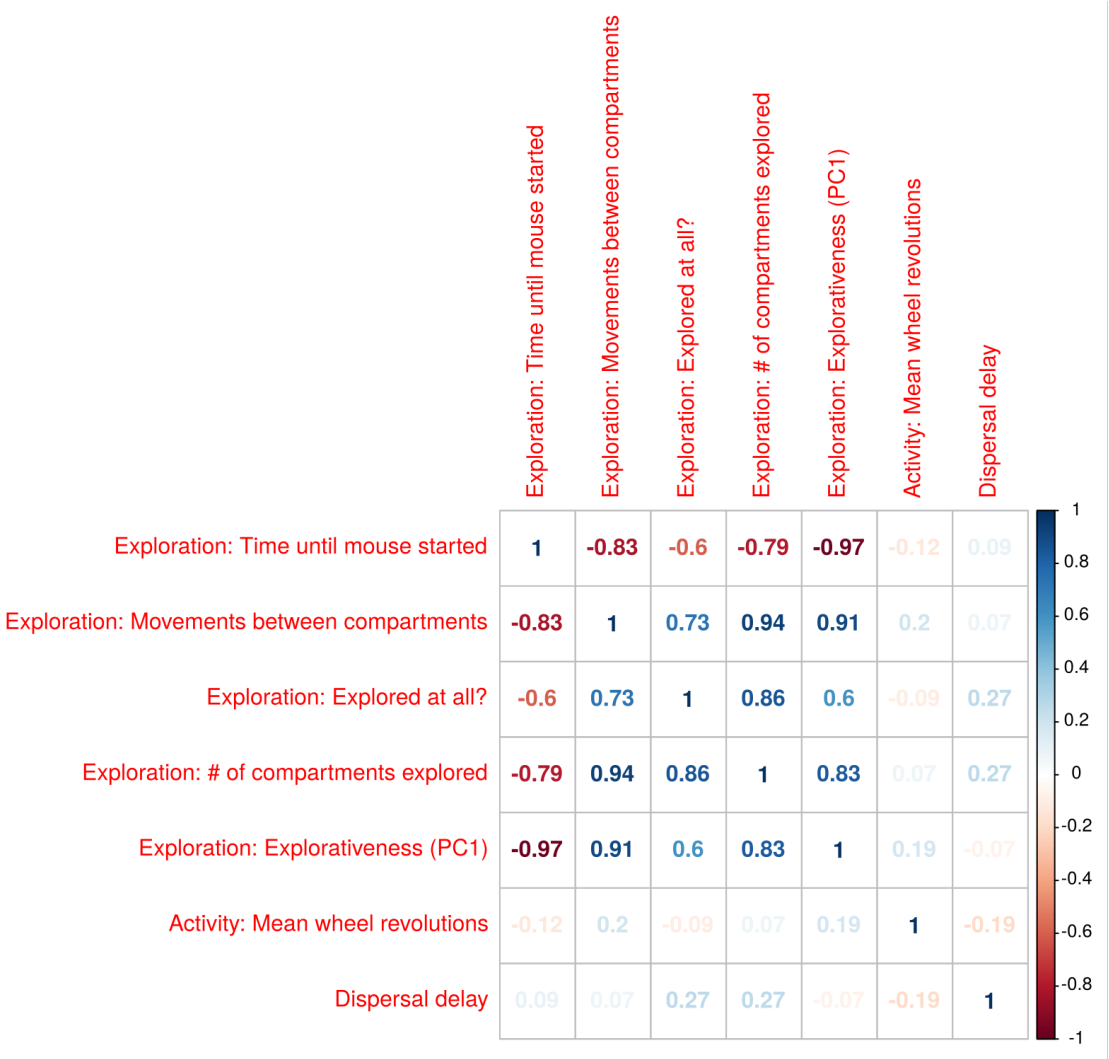


Figure 4.9: Pearson correlation matrix of dispersers who were tested for exploratiiveness ($n = 21$) with four components of exploration and the combined measure exploratiiveness, activity measured in mean wheel revolutions, and dispersal latency (in days).

tional long-term study (Runge and Lindholm 2018) and computer simulations (Chapter 3), this study constitutes the third piece of evidence that this effect exists. We therefore conclude that the effect is real, which makes the *t* haplotype the first selfish genetic element to have been shown to impact dispersal behavior. However, we were unable to find strong evidence that the increase in dispersal propensity of +/*t* is primarily seen in higher densities, an effect that was seen in the long-term study and predicted by simulations. The raw number of +/*t* dispersers was indeed much higher than that of +/+ dispersers in higher densities and only slightly higher in lower densities, but this effect was not statistically significant. In general, males were much more dispersive than females, which is not what we found in the long-term study, in which females were a bit more dispersive than males (Runge and Lindholm 2018), but both effects have been found in house mice (reviewed in Pocock et al. (2005)).
Surprisingly, we also found that heavier +/*t* mice were more likely to disperse than +/+ mice. We did not hypothesize this a priori and so we consider this an exploratory result. We did not find this effect in the previously analyzed long-term study on free-living house mice (Runge and Lindholm 2018), in which we were, however, only able to test for an impact of the body weight as a pup rather than weight as a sub-adult as we did here. However, we did find that +/*t* pups were heavier, in Runge and Lindholm (2018), and we also find a slightly increased sub-adult weight in the present study. In house mice, male dominance status is positively predicted by body weight (DeFries and McClearn 1970) and subordinate males are evicted from dominant males and thus more likely to disperse (Gerlach 1990), but there is no evidence that +/*t* are more likely to be dominant, but one study found +/*t* males to be less successful at holding territories (Carroll et al. 2004). Put together, no clear picture regarding +/*t*'s dominance emerges. Studies on other species found more positive than negative effects of body weight on dispersal propensity (Clobert et al. 2009). In side-blotched lizards, the effect of an individual's weight as an egg depended on their genetic background (Sinervo et al. 2006). In naked mole-rats, distinct dispersal morphs are bigger than their resident conspecifics (O'Riain et al. 1996), possibly to mitigate costs of dispersal (Bonte et al. 2012). Therefore, the effect of body weight on dispersal appears can be of two extremes (Bonte and Peña 2009; Kisdi et al. 2012): are dispersers of high condition and thus presumably

with increased odds of successful dispersal? Or are they of low condition because they are drawn from the pool of those that failed to establish themselves as residents? From our results, it appears that in house mice this could depend on whether a mouse carries the *t* genotype: Genetically prone-to-disperse +/*t* could increase odds of successful dispersal if they remain prone to disperse at higher body weights—and perhaps have increased body weights to begin with—while heavier +/+ may be rewarded with increased chances of successfully establishing as dominant residents if they do not disperse. Further work on this novel and unexpected effect will be needed.

We found that +/*t* showed more exploratory behavior than +/+, which is one of the behavioral traits hypothesized to be part of dispersal syndromes (Ronce and Clobert 2012), but exploration is also sometimes found to be decreased in dispersers, such as in deer mice (Fairbairn 1978). Otherwise, dispersal events could be preceded by bouts of exploration, in which the disperser-to-be searches for new territories, as is the case in deer (Debeffe et al. 2013) and squirrels (Haughland and Larsen 2004). Thus, being more prone to exploratory behaviors could be beneficial for those who are prone to disperse, such as +/*t*. However, a previous study on adult +/*t* under different experimental conditions did not find a statistically significant difference in exploratory behavior with the mean being elevated in +/*t*, but overlapping +/+ in its distribution (Auclair et al. 2013). Combined with our results, this could suggest that behavioral differences of +/*t* and +/+ that are connected to dispersal may be found primarily in juveniles. Males and females did not differ in their exploration behavior, similar to what was found by Vošlajerová Bímová et al. (2016). In accordance with their conclusions, this lack of difference did not predict that males and females would not differ in dispersal.

Activity levels in the wheel running experiment very clearly did not differ between +/+ and +/*t*. This is in contrast to our hypothesis, but is interesting with regards to distinguishing how +/*t* differ from +/+, because the exploration experiment—being essentially a modified open field test—could have been interpreted as measuring activity (Stanford 2007). However, it is important to note that previous experiments on adult mice, using an empty cage rather than running wheels, found that adult +/*t* females were less active than their +/+ conspecifics (Auclair et al. 2013), which lined up with differences in food consump-

tion (Auclair et al. 2013), resting metabolic rates (Lopes and Lindholm 2020), and lifespan (Manser et al. 2011). We speculate that the difference in age between the mice in our experiment and the mice of previous studies could play a role, but experiments using the running wheel and adult females will be necessary to disentangle the effects of age and experiment setup. Interestingly, lines of mice artificially selected for high activity in running wheels showed no difference to control lines in activity outside the running wheel (Koteja Paweł and Garland Jr. et al. 1999), putting into question the relation between different forms of activity, which could also explain the disparity between different studies on +/*t* mice. Furthermore, those selected lines also did not differ from control lines with regards to their behavior in open-field test (Bronikowski et al. 2001), whereas we found that activity in the running wheels did correlate with the number of movements between compartments in the exploration experiment, which we propose would be the most comparable measure to classic open-field test movement measures. Females were found to turn the running wheels much more often than males, which has been found by others as well (Lightfoot et al. 2004; Bartling et al. 2017).

We found that +/*t* are more dispersive and more prone to explore, but why did we not find a strong correlation between dispersal and the tendency to explore? Environmental cues play a major role in the individual decision to disperse (Ronce 2007), as we can also see in the influence of density on dispersal in our study on the long-term population (Runge and Lindholm 2018) and in birds and mammals more generally (Matthysen 2005). The pattern that we find, one of a weak, insignificant, positive correlation between dispersal and exploration, is consistent with the idea that the tendency to explore could be one of many traits that, in combination with environmental cues, make up the decision to disperse.

Carrying the *t* haplotype increased the propensity of mice to explore and disperse. Its carriers had an increased body weight and were more prone to disperse at higher body weights than +/+. Put together, the *t* haplotype appears as a genetically based dispersal polymorphism. It is important to remember that the *t* haplotype is expected to spread very rapidly in populations of low density and low *t* frequency due to the maximized effect of its drive and minimized impact of its deleterious traits (poor sperm competition and lethal ho-

mozygosity)(Levin et al. 1969). Thus, *t* is not just increasingly dispersive, but should also be particularly successful once a +/*t* immigrates into a well-suited population. In summary, the *t* produces a dispersal phenotype in its carrier, a phenomenon rarely observed in mammals, otherwise only known from naked mole-rats (O'Riain et al. 1996). In doing so, it may avoid the expression of its deleterious traits and increase the expression of its advantageous traits (Chapter 3), which, all in all, makes a good case for increased dispersal of +/*t* being selected directly rather than a by-product (see Burgess et al. (2016)). It will be interesting to see whether other meiotic drive systems with similar fitness (dis-)advantages, such as *SR* in *Drosophila* (Price et al. 2008), also show differences in their dispersal phenotypes.

4.6 Acknowledgments

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4.7 Author contributions

JNR and AKL conceived the study and wrote the manuscript. JNR analyzed the data and conducted the majority of the experimental work.

4.8 Ethics

The experiments were carried out under the permits ZH075/18 and ZH134/16 of the cantonal authorities in Zurich, Switzerland.

5 Towards the genetic basis of dispersal: cost-efficient whole-genome imputation

Germaine Léa Bongenge

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5.1 Introduction

Dispersal describes the movement of an individual from its population and place of birth to a new population with subsequent breeding. These movements contribute to gene flow (Bohonak 1999) and influence mating opportunities (Perrin and Mazalov 1999). Dispersal is thus very consequential for individual fitness and species survival, for example with regards to climate change (Thomas et al. 2004). But while there are advantages for successful dispersers, these come at high risks—including death—for those who do not succeed (Bonte et al. 2012). The expression of dispersal is therefore predicted and usually found to depend on external and internal cues for the potential disperser (Ims and Hjermann 2001; Matthysen 2005; Kisdi et al. 2012). However, there is increasing evidence that dispersal is also (in part) predicted by genetic variation (Saastamoinen et al. 2018), i.e. there are individuals in a population that are more or less prone to disperse based on their genetics. Nonetheless, dispersal is a highly complex trait that is shaped by a plethora of evolutionary forces (Matthysen 2012). It can be separated into three key steps—emigration, transfer, and immigration (Clobert et al. 2009)—each presumably with its own genetic components. It is usually found to be polygenic (based on many loci), which makes responsible loci harder to detect (Saastamoinen et al. 2018). Some dispersal may not even be selected directly, but rather emerge as a consequence of other traits (Burgess et al. 2016), which could further obfuscate truly causal loci. Furthermore, the environmental- and condition-related causes of dispersal need to be accounted for. Hence, the genetic basis of dispersal is difficult to study and remains poorly understood.

In the previous chapters, we described a genetically-based dispersal polymorphism in house mice, the *t* haplotype. The *t* haplotype is primarily a 35 Mb large meiotic driver in house mice that increases its transmission to offspring of male carriers (Burt and Trivers 2006; Lindholm et al. 2016, 2019; Kelemen and Vicoso 2018). However, we also found it to increase dispersal and dispersal-related traits (Chapters 2, 3, and 4). Based on this finding and an unpublished preliminary analysis that showed that dispersal is a heritable trait in this population, we are now aiming to uncover the genetic basis of dispersal in the same study population more broadly by asking the following questions: Is there vari-

ation on the *t*-carrying chromosome that predicts dispersal in mice that do not carry the *t* haplotype? Are other loci interacting with the *t* haplotype to modify dispersal behavior? What other loci are in general involved in dispersal?
To uncover the genetic basis of any trait in a big population, one needs a technological approach with reasonable costs (Sampson et al. 2011). While whole genome sequencing is needed to uncover all genotypes of an individual, genotyping only at pre-selected loci using SNP arrays has long been the method of choice with regards to cost efficiency (Vignal et al. 2002). However, this method is limited to species or populations for which a well-working SNP array exists. In other cases, researchers could opt to create a new SNP array, which would come at increased costs. Recently, new statistical methods have allowed for another, much cheaper, option: whole-genome imputation (Davies et al. 2016). Imputation describes a process by which genotypes are statistically inferred rather than directly observed. It is possible to impute genotypes when some genotypes are known through other means and the haplotypes—regions of linked genotypes—are known in the population (Pasaniuc et al. 2012). Then, the presence of observed alleles can be used to predict the presence of unobserved ones. Typically, haplotype maps with detailed information on the haplotypes in the population in combination with already rather informative SNP arrays are used to impute genotypes at even more loci (Marchini and Howie 2010). In our case, we cannot rely well on available SNP arrays, because they are made primarily for lab mice (Morgan et al. 2016), which differ drastically from wild mice (Salcedo et al. 2007). We do not have detailed haplotype maps for the study population either. However, we have access to DNA samples of the twelve mice that founded the study population that forms the basis of our work and have so far never found evidence of immigration into the study population. Therefore, all mice that ever lived in the population can be assumed to be genetic mosaics of the founders in the population. Now, novel statistical methods (Corbett-Detig and Nielsen 2017) allow us to infer what regions of the genome of a mouse were inherited from what founder, which we then convert into genotypes, thereby imputing almost the whole genome.
Here we present the validation and methodology behind our adaptation of the statistical inference of ancestries using *AncestryHMM* (Corbett-Detig and Nielsen 2017) into whole-genome imputation in a founder population of free-

living wild house mice.

5.2 Methods

We used *R* 3.5.1 (R Core Team 2018) with *ggplot2* (Wickham 2009) for visualization and analysis.

5.2.1 The population

We are studying an intensively monitored population of free-living wild house mice that has been intensively monitored since 2002, when it was founded using twelve mice from two nearby source populations (König et al. 2015). The mice live in an old barn, which they can freely leave and re-enter. Food and water is provided *ad libitum*. The barn is regularly searched for new litters and the mice are genetically sampled and genotyped at 25 microsatellite loci when they are 13 days old and again as adults (≥ 17.5 grams of weight) and when they are found dead. The genotypes are used for identification (e.g. which pup became which adult) and pedigree construction.

5.2.2 Sequencing

We sequenced the twelve founders (F_0) of the population and eight F_1 offspring of them, at least one for each founder, with on average 9.24x (SD=1.16) coverage, i.e. each base was on average sequenced 9 times. We used the Illumina Hi-Seq platform, which produces paired-end reads of 150 base pairs. The coverage given excludes duplicated sequences. These were also excluded from all subsequent analyses by marking them as duplicates.
We then sequenced 1,634 individuals from later generations, F_x, on the Illumina NextSeq platform with single-end 75 base pair long reads at on average 0.018x coverage (SD=0.01). We excluded individuals that were sequenced at less than 0.005x coverage from further analyses, as their DNA quality was likely subpar (see Figure 5.1). Subsequently, 1,428 individuals remained.
We aligned all reads (F_0, F_1, and F_x) to the reference house mouse genome (GRCm38.p6) using the *mem* algorithm of bwa 0.7.17-r1188 (Li 2013), which were

then sorted and duplicates marked using Picard toolkit 2.18.26 (Broad Institute 2019) (see Listing 5.1).

Listing 5.1: Aligning the founder genomes

```
1 bwa mem -r 1.5 -E 1 -w 100 -T 0 -a reference_genome.fasta
     forward_reads_file.fastq.gz reverse_reads_file.fastq.gz
     > aligned_genome.sam
2 picard SortSam I=aligned_genome.sam O=sorted_genome.bam
     SORT_ORDER=coordinate
3 picard MarkDuplicates I=sorted_genome.bam O=final_genome.
     bam TAGGING_POLICY=All
```

5.2.3 Founder genotypes

To accurately impute genotypes of the F_x, we first needed to generate the F_0 haplotypes that founded the population and from which all following generations were mosaics of. We called variants using bcftools 1.9 (Li 2011) and Strelka 2.9.10 (Saunders et al. 2012), independently (Listings 5.2 and 5.3). We filtered both sets of variants to only include loci at which at least 50% of individuals had a genotype with genotype quality GQ ≥ 45 and read depth DP ≥ 3 (Listing 5.4), and from then on only used variants that were present in both using the function *isec* of bcftools. Furthermore, we only included genomic regions that had a good mapping quality MQ ≥ 20 and on average not much higher coverage COV $\leq$ 25x or lower coverage COV $\geq$ 5x than expected (which may be evidence of regions that are difficult to align to the reference genome or of copy number variants). Finally, we excluded all loci where at least one F_0-F_1-parent-offspring-trio had an incongruent genotype ("Mendelian errors", e.g. two A/A parents with an A/G offspring), calculated using the function *mendel* of VCFtools 0.1.16 (Danecek et al. 2011). After all these steps, we discovered 1,369,409 autosomal loci at which there was variation within the founders ("the loci of interest"). Note that all following procedures exclude non-autosomal loci, which will be included in future improvements to the protocol.

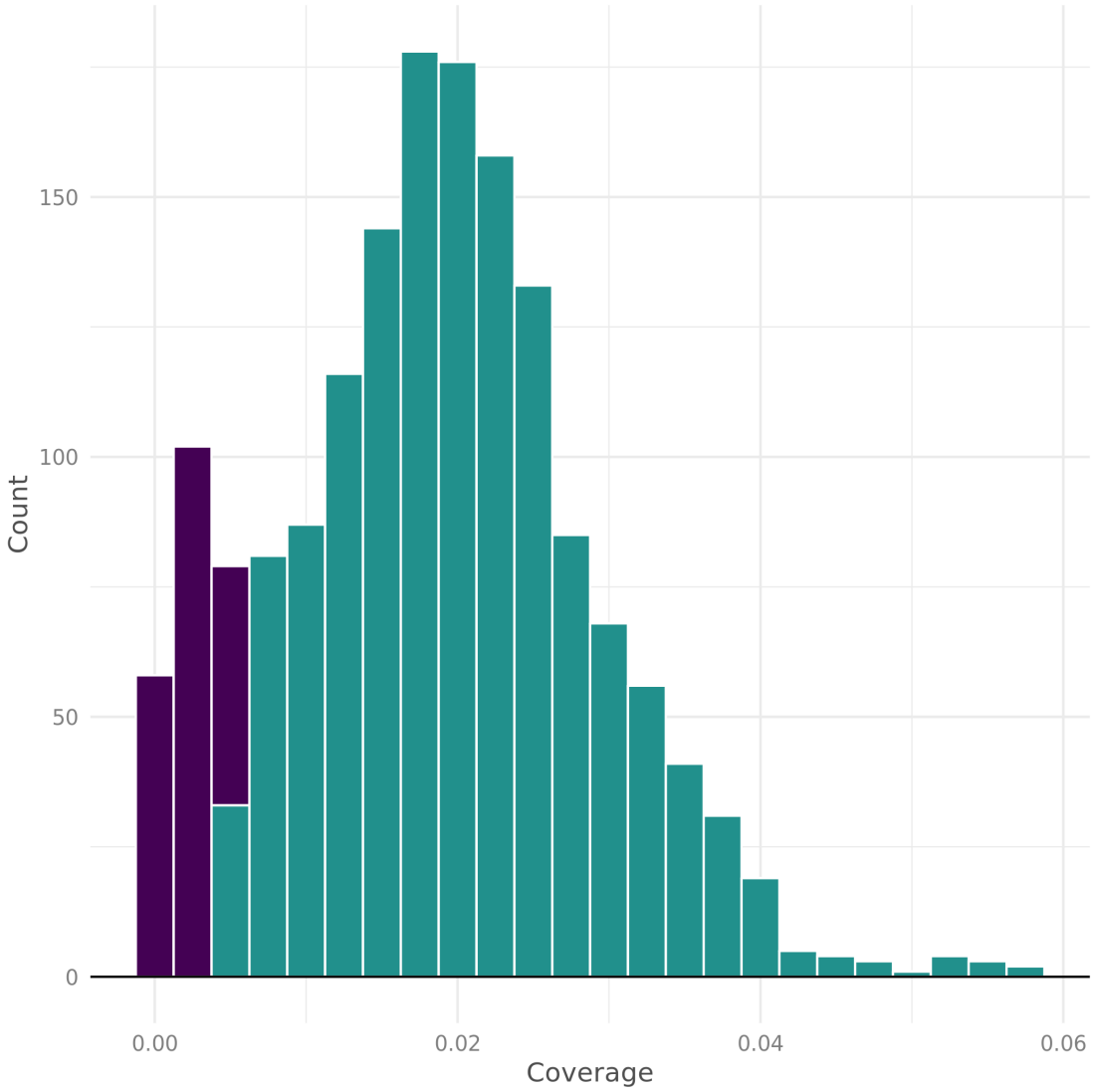

Figure 5.1: Overview of the ultra-low coverages at which the 1,634 mice of later generations were sequenced. Green represents those above 0.005x that were included in subsequent analyses.

Listing 5.2: Calling variants using bcftools

```
bcftools mpileup -a FORMAT/AD,FORMAT/DP,FORMAT/SP,INFO/AD
    --output outputfile.mpileup -Ou -f reference_genome.
    fasta f0f1_1.bam f0f1_2.bam ... f0f1_n.bam
bcftools call -mv -Ob -o variants.bcf -f GQ outputfile.
    mpileup
bcftools sort --output-type b --output-file variants.sorted
    .bcf variants.bcf
```

Listing 5.3: Calling variants using Strelka

```
configureStrelkaGermlineWorkflow.py f0f1_1.bam f0f1_2.bam
    ... f0f1_n.bam --referenceFasta reference_genome.fasta
    --runDir output_directory/
output_directory/runWorkflow.py -m local
```

Listing 5.4: Filtering variants

```
vcftools --bcf bcftools-variants.sorted.bcf --max-missing
    0.5 --minQ 100 --minDP 3 --minGQ 30 --recode-bcf --
    recode-INFO-all --out bcftools-variants.sorted.filtered
vcftools --gzvcf strelka-variants.vcf.gz --max-missing 0.5
    --minQ 100 --minDP 3 --minGQ 20 --recode --recode-INFO-
    all --out strelka-variants.filtered
```

Genotypes alone would be insufficient for accurate imputation, because alleles are not transmitted randomly, but rather as haplotype regions, which is what the imputation algorithm makes use of. Thus, in order to determine which alleles are on the same chromosome (of each pair) and which are therefore more likely to be transmitted together via linkage, we used a process called phasing. In this case, we used the F_0 and F_1 genotypes and their known pedigree to determine which alleles were transmitted together, thereby inferring whether they are on the same chromosome. This was done using WhatsHap 0.18 (Martin et al. 2016) (Listing 5.5).

Listing 5.5: Phasing with WhatsHap

```
whatshap phase --ped pedigree.ped -o phased_variants.vcf
    variants.vcf f0f1_1.bam f0f1_2.bam ... f0f1_n.bam
```

5.2.4 Preparing the *AncestryHMM* input files

Next, we used *AncestryHMM* (Corbett-Detig and Nielsen 2017) to detect for each mouse in the F_2 and following generations (F_x) which parts of its genome were inherited from what founder. Below, we describe the steps to create the input files for *AncestryHMM*.

5.2.4.1 Founding ancestries

The phased genotypes of the 12 founder mice were divided into 24 ancestral haplotypes for each autosome (each founder contributing two haplotypes based on the two homologous chromosomes). We used *mpileup* of samtools 1.9 (Li et al. 2009) to count the number of and which bases were read at the loci of interest in each of the founders. *AncestryHMM* requires as input the number of times the reference and alternative base was present in each ancestry at each locus. In phased or homozygous genotypes, we combined the number of times a base was read with the genotype that was called in each haplotype to include how sure of the call we were into the model. For example, a heterozygous phased genotype that was called in one founder as *A* (here, the reference base) in one haplotype and *G* (the alternative base) in the other, with the bases having been read 6 and 5 times, respectively, would be coded as seen in row 1 of Table 5.1, with other examples given as well. In case of a genotype that is heterozygous but could not be phased successfully, we put the same allele counts into both haplotypes.

Table 5.1: Examples of four loci of one founder (F_0) being coded for *AncestryHMM*, each with 11 sequenced bases. The first column indicates the genotype of the locus that is being coded and columns 2-5 show what would be written into the input file in each case.

Genotype example	haplotype 1, ref. alleles	haplotype 1, alt. alleles	haplotype 2, ref. alleles	haplotype 2, alt. alleles
Heterozygous, phased	6	0	0	5
Heterozygous, not phased	6	5	6	5
Homozygous reference	11	0	11	0
Homozygous alternative	0	11	0	11

5.2.4.2 Genetic map

AncestryHMM requires a genetic map, i.e. the probability of recombination between loci, to calculate the breakpoints between ancestries in the F_x. While there are empirical studies that have generated genetic maps in house mice, these have been done on lab strains (Cox et al. 2009; Liu et al. 2014), which differ significantly from our mice, even in number of physical chromosomes (Grize et al. 2019). We thus decided to use recombination probabilities estimated from the physical distance between loci (10^{-6} Morgans per base).

5.2.4.3 Incorporating later generations

For the later generations, we used *mpileup* to count the bases and which bases were read at the loci of interest. Because we usually read only one base at each locus that we sequenced in the F_x, we only used bases with a base quality score of $BQ \geq 30$ to not bias the results. We then placed the number of reference and alternative alleles read for each F_x into the table to complete the *AncestryHMM* input file.

5.2.5 Calculating ancestry proportions

We used *AncestryHMM* to calculate the probabilities for each ancestry combination (for example heterozygous for founder haplotype 17 and founder haplotype 23) at all loci of interest for which we had reads of an F_x. We assumed an equal probability for all ancestries, a genotyping error rate of 0.1%, and the number of generations that passed since F_0 were assumed to be one per year since the founding of the population until the DNA sample was taken for each F_x, but this assumption was not fixed, i.e. *AncestryHMM* was allowed to deviate from it (Listing 5.6). Manser et al. (2011) calculated a generation time of 9 months, but in the earlier years of the study where density was much lower and, presumably, competition over reproduction was less strong, therefore we chose 12 months as an estimate and will refine this further. Next, we tried to infer the ancestry probabilities at all loci of interest. For each ancestry probability in between two F_x sequenced loci, the probability would be

$$P_i = P_{D_i} + (P_{U_i} - P_{D_i}) \cdot s_{i,D_i,U_i}$$

with i being the focal locus, D_i being the closest sequenced locus downstream and U_i being the closest sequenced locus upstream, and s being the proportion of the genetic distance between D_i and U_i that the locus i is located at. With this method, we inferred ancestry proportions at all loci of interest with the exception of the loci that preceded and succeeded, respectively, the first and last F_x sequenced loci on each chromosome, because we did not want to assume that there was no recombination event before and after the first/last sequenced locus.

Listing 5.6: Running *AncestryHMM* with individualized number of generations and 24 ancestries with the same assumed proportions (1/24 or 4.2 per cent)

```
1 ancestry_hmm -i input_file -a 24 0.0417 ... 0.0416 -p 0 -{
    generations} 0.04 ... -p 23 -{generations} 0.04 -s
    diploid_sample -e 0.001
```

5.2.6 Inferring genotypes

Next, we inferred genotypes from the ancestry probabilities. We took advantage of the fact that *AncestryHMM*'s probabilities at each locus add up to one. We summarized the probabilities for each ancestry by taking the probability for this locus to be homozygous for ancestry y and adding half of the sum of all probabilities for this locus to be heterozygous for ancestry y, because the probability to be heterozygous represents the probability for only one chromosome (per locus) to descend from this ancestry.

$$\sum P_y = P_{y_{\text{Hom}}} + 0.5 \cdot \sum P_{y_{\text{Het}}}$$

For example, if *AncestryHMM* was sure that the focal individual at one locus is heterozygous for ancestry y, but completely unsure about which of the other 23 ancestries is the other one, except that it is not y, then the summarized probability for ancestry y would be $\sum P_y = 0.0 + 0.5 \cdot (23 \cdot \frac{1}{23})$, which is 0.5, which represents either heterozygosity for ancestry y or 50% probability to be homozygous for ancestry y, which makes no difference to the next step.
We multiplied this total probability P_y for ancestry y at each locus with the allele of this ancestry (reference allele = 0.0, unphased = 0.5, and alternative allele = 1.0). We did this for all 24 ancestries and then summed these allele values up at each locus to receive an imputed proportion of the alternative allele for each locus of each offspring (between 0 and 1). Imputed proportions between 0.0 and 0.1 were inferred as a homozygous reference allele genotype, between 0.45 and 0.55 as heterozygous, and between 0.9 and 1.0 as homozygous alternative allele. For an example distribution of imputed allele proportions, see Figure 5.2.

5.2.7 Determining the quality of the imputation

After having imputed the genotypes, we set out to quantify how well the imputation worked. We used five approaches.
First, we quantified the proportion of loci that were imputed as a function of the coverage that we had for each individual. Whether the plot of all individuals in this regard would appear to reach an asymptote would inform us about whether more coverage would increase the number of loci imputed or whether

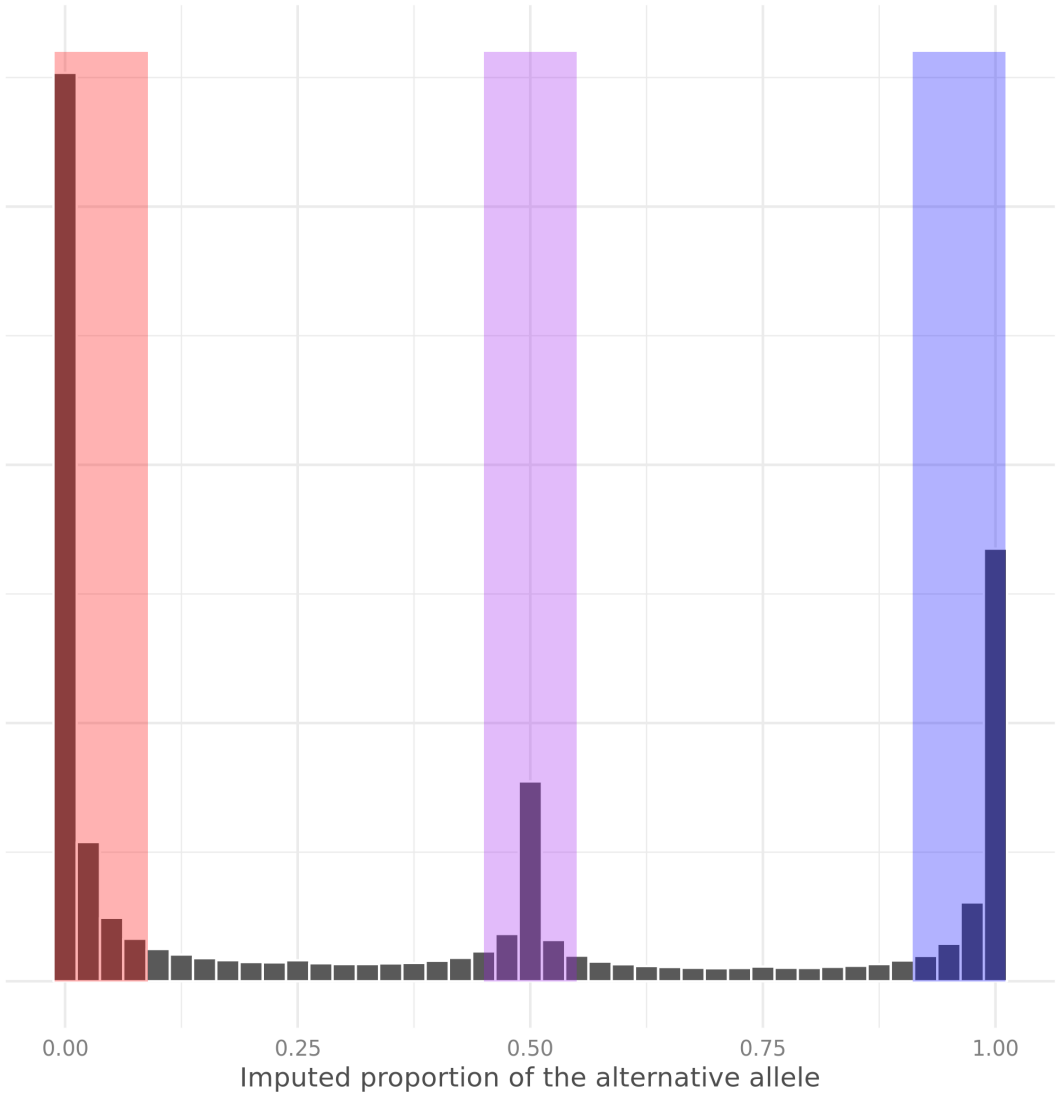

Figure 5.2: An example distribution of the imputed proportion of the alternative allele with cutoff areas highlighted (red = homozygous reference allele, purple = heterozygous, and blue = homozygous alternative allele).

we had already reached a good compromise between costs of sequencing more coverage and the percentage of loci that we were able to impute. To describe the gains of loci imputed with increasing coverage more precisely, we created a linear model of the number of loci imputed with the logarithm of the coverage as a predictor using *R* 3.5.1 (R Core Team 2018).
Second, we compared the imputed genotypes of 12 individuals for which we also had genotypes collected using the commercially available SNP array *GigaMUGA* (Morgan et al. 2016). Without any further filtering, *GigaMUGA* scored up to 132,210 loci in our sample. Of these, only 7,434 are variable in the F_0 of our population, leaving at least 99.5% of the *GigaMUGA* loci uninformative for our purposes while costing more than 6 times as much as our approach, which illustrates why we chose to do whole-genome imputation. For our *GigaMUGA vs.* imputation comparisons, we only included loci that were variable within the individuals genotyped in *GigaMUGA* and fulfilled basic quality criteria by removing loci that were missing in more than one-fourth of the samples or heterozygous in more than half of the samples, which are recommended variables to filter by (Morgan 2016). We looked at the proportion of loci genotyped differently between the two methods (the discordance) using VCFtools as a function of the coverage for the same reason as laid out above (Listing 5.7).

Listing 5.7: Calculating genotype discordance with vcftools

```
1 vcftools --vcf Imputed.vcf --out outfile --diff GigaMUGA.
    vcf --diff-indv-discordance
```

Third, we generated identity-by-descent (IBD) measurements using TRUFFLE 1.38 (Dimitromanolakis et al. 2019) for all combinations of individuals (in dyads), i.e. we calculated how much of their genome they share from the same recent common ancestor (Listing 5.8). We compared those measurements with the pedigree of the population that was generated previously using microsatellites and information of what individuals could have been parents (as used in Ferrari et al. (2019)). We further compared the IBD measurements of parent-offspring and full sibling relationships as a function of coverage to better understand which individuals were imputed well and which ones were not.

Listing 5.8: Calculating IBD measurements with TRUFFLE

```
truffle --vcf Imputed.vcf --maf 0.1 --missing 0.2 --
    segments --out truffle_output
```

Fourth, we looked at the imputed genetic variation of chromosome 17, on which some individuals in the population carry the *t* haplotype, the large and unique variety of the proximal third of chromosome 17 analyzed in Chapters 1, 2, and 3. We highlighted the carriers of the *t* haplotype in a PCA of the imputed variation to see whether the imputation algorithm correctly inferred the presence of a distinct haplotype in carriers of the *t*. We computed the PCA using SNPRelate 1.16.0 (Zheng et al. 2012) with R 3.5.1 (R Core Team 2018).
Fifth, we quantified the number of loci that, given their genotypes, could not have been transmitted via a parent-offspring relationship ("Mendelian errors", see Founder genotypes). Mendelian errors are computed in (hypothetical) parent-offspring-trios. We first selected the 50 most closely related individuals based on kinship inference from IBD measurements. For these, we computed Mendelian errors in all possible parent-offspring-trio permutations. Then, we selected the lowest Mendelian error rate that two focal individuals had with each other of all the parent-offspring-trios that involved them, the "minimum Mendelian errors". Then, we divided the number of errors by the amount of loci that were imputed in both, getting a proportion of loci with Mendelian errors. We also used Mendelian errors to quantify imputation errors as a function of coverage using parent-offspring dyads, who should have 0 errors, except for mutations.

5.3 Results and Discussion

We were able to impute on average 78.8% (SD=11.7%.) of the 1,369,409 loci of interest (Figure 5.3). Based on the distribution of imputed loci against the coverage at which individuals were sequenced, we determined that the current range of coverages (0.02x to 0.03x) is already a good loci-per-coverage compromise. According to a linear model of the loci imputed per logarithm of coverage, we found that at a coverage of 0.005, an increase in coverage by 0.005 increases the

number of loci imputed by 11.3%, at 0.010 by 5.9%, at 0.015 by 4%, at 0.020 by 3%, at 0.025 by 2.3%, at 0.030 by 1.9%, 0.035 by 1.6%, and at 0.040 by only 1.4%. We compared the imputed genotypes of twelve mice with a commercial SNP array and found a mean of 8.3% (SD=1.8%.) loci to be discordant. Based on visual inspections, this value appears to decrease with coverage (Figure 5.4). However, we did not have any individuals at 0.03x coverage to determine whether imputation works much better at that point.

The first two dimensions of a PCA of genetic variation in the imputed genotypes of all autosomes clearly show a difference between carriers of the *t* haplotype, which have a unique variation of 35 Mb on chromosome 17, and non-carriers, as determined beforehand using genetic markers (Figure 5.5). Two points stand out as being apparent wildtype carriers within the *t*-carriers. Subsequent retyping of these two individuals confirmed that they are indeed *t*-carriers, underscoring the accuracy of our whole-genome imputation approach.

We compared identity-by-descent (IBD) measurements calculated from the imputed genotypes with four kinds of pedigree-based relationships: parent-offspring, full sibling, duplicate samples, and other relationships. In Figure 5.6, we can see that the more closely two samples are related (from up to down), the proportion of the genome that is detected as IBD in at least one chromosome increases. In general, the expected values would be 1.0 for duplicates who share all chromosomes, 1.0 for parent-offspring who share one copy of all chromosomes, 0.5 for full siblings who either share 0, 1, or 2 chromosomes at all loci, thus on average 0.5, and 0.0 for other relationships. However, we found that this is likely a highly inbred population, with the first F_0 parents of the F_1 unknowingly at least in part made up of closely related individuals already and thus values that should be < 1 are shifted closer to 1. The number of segments that are identical-by-descent (the y axis in Figure 5.6) were not as differentiated between the different relationships, but full siblings and parent-offspring had mostly fewer segments than the less related individuals and duplicate samples had markedly decreased numbers of segments. Usually, this number would help distinguish these relationship categories, with more distantly related individuals having higher numbers of segments, because more recombination has taken place, decreasing the size of segments and thereby increasing the number of segments (if the proportion of genome that is IBD remains unchanged).

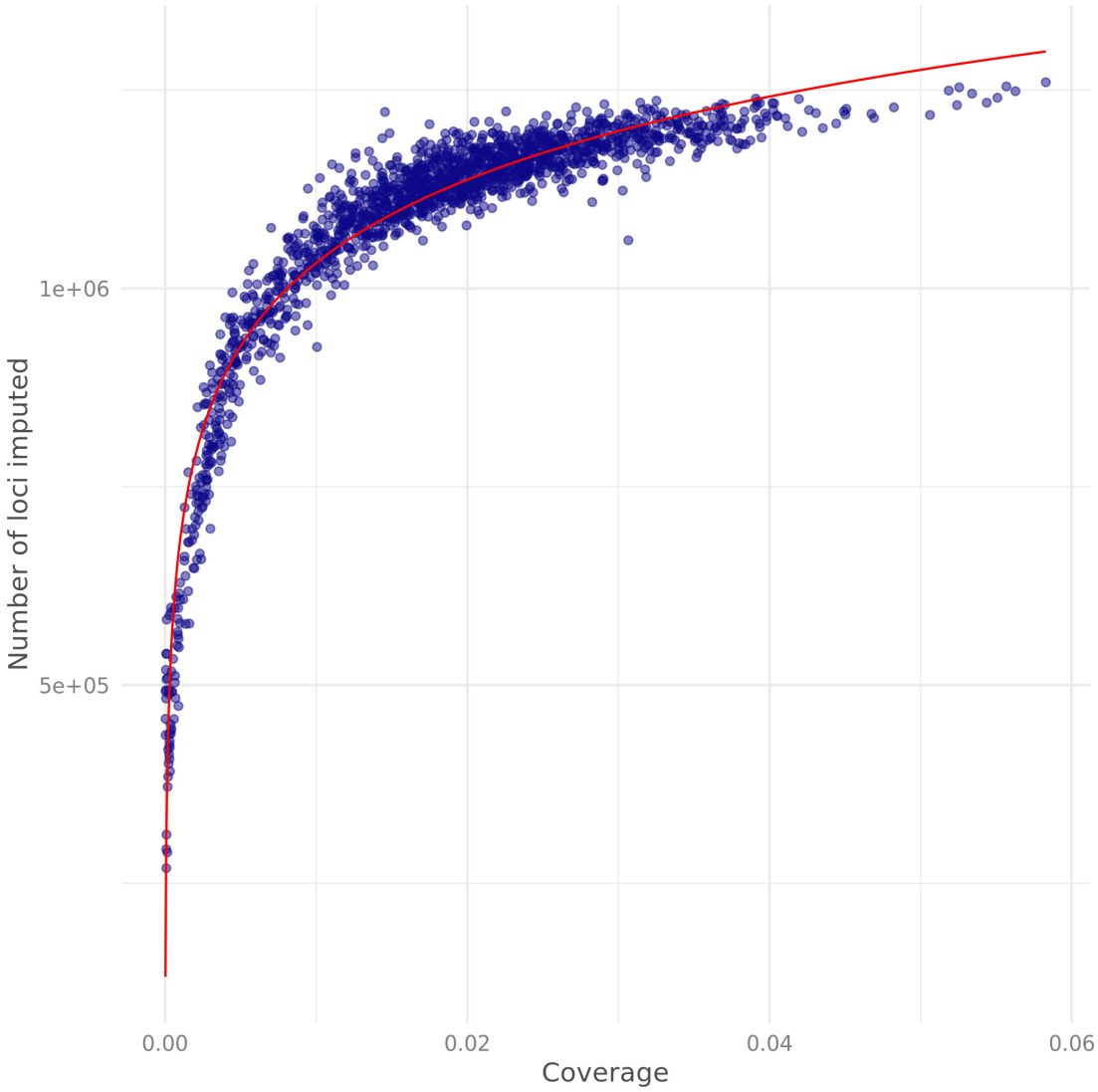

Figure 5.3: The number of loci that were imputed plotted against the coverage with each dot representing an individual that was imputed successfully ($n = 1634$). The red line indicates the prediction of a linear model of loci imputed $\sim$ log(coverage).

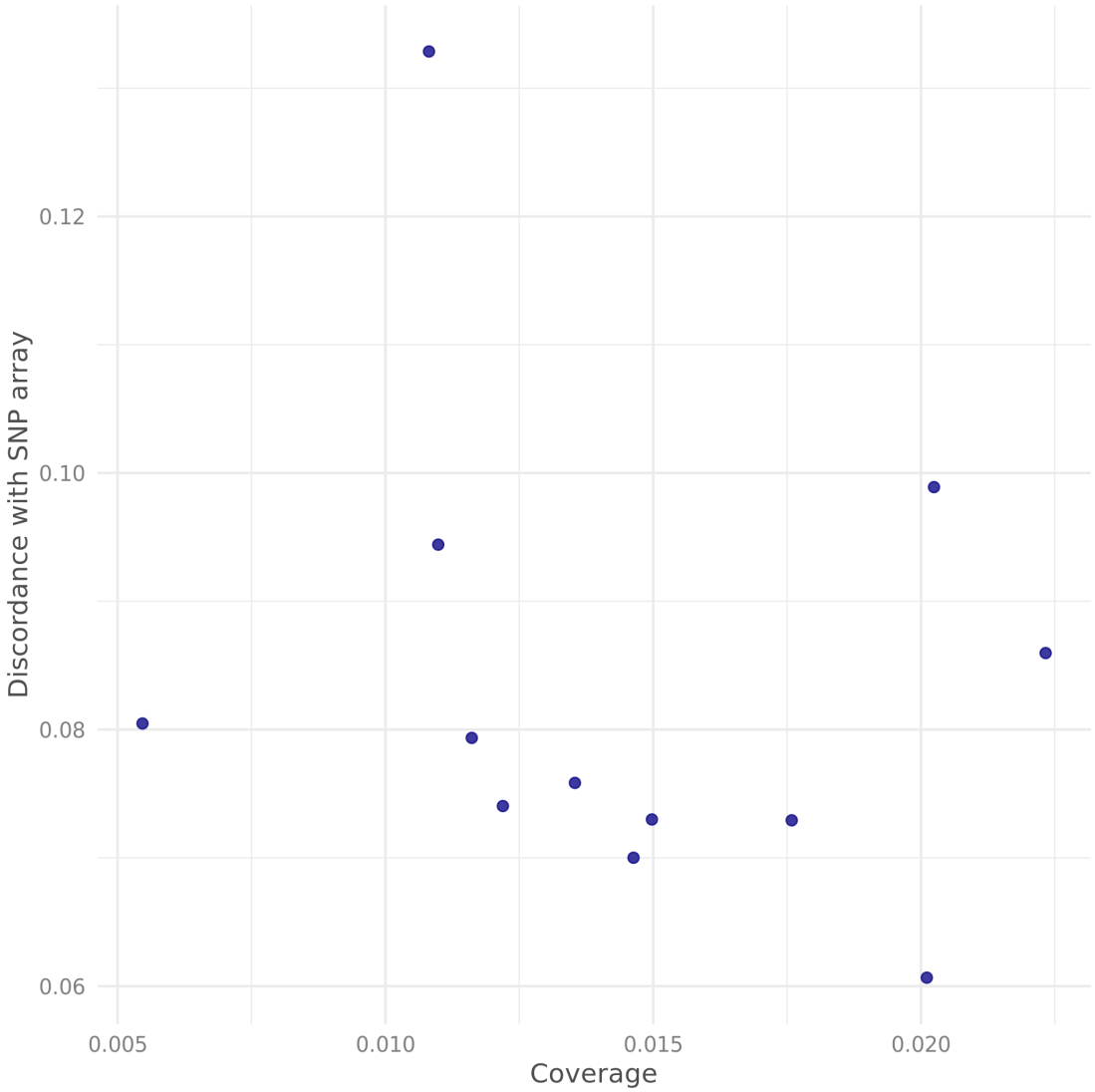

Figure 5.4: The proportion of genotypes that are called differently between a commercial SNP array and our imputation approach for 12 individuals with on average 1044.7 loci overlapping.

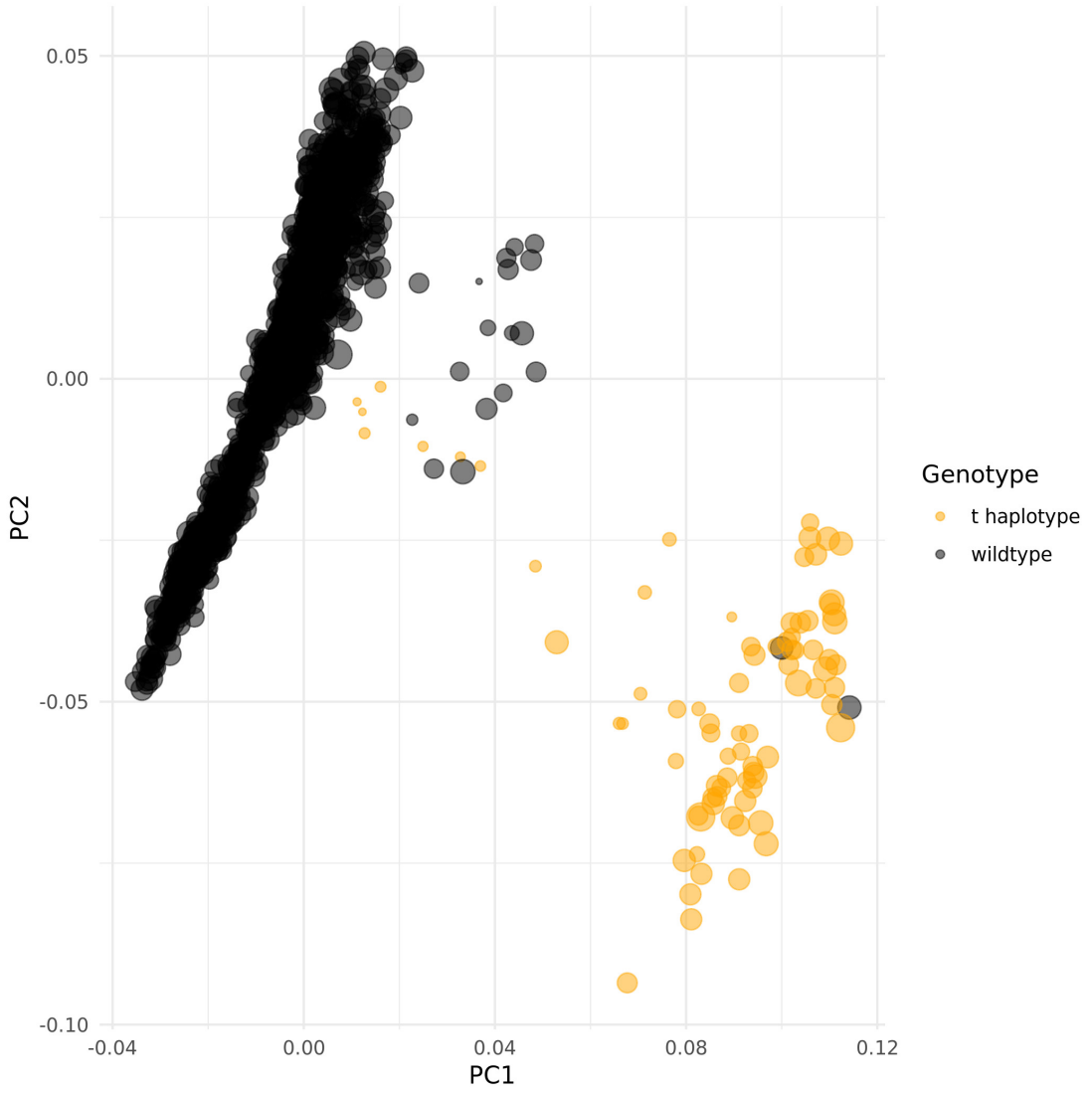

Figure 5.5: PCA of the genetic variation in the imputed F_x genotypes of all autosomes. Orange highlights carriers of the *t* haplotype. The size of each dot is scaled with the coverage, with larger equaling higher coverage ($n = 1634$).

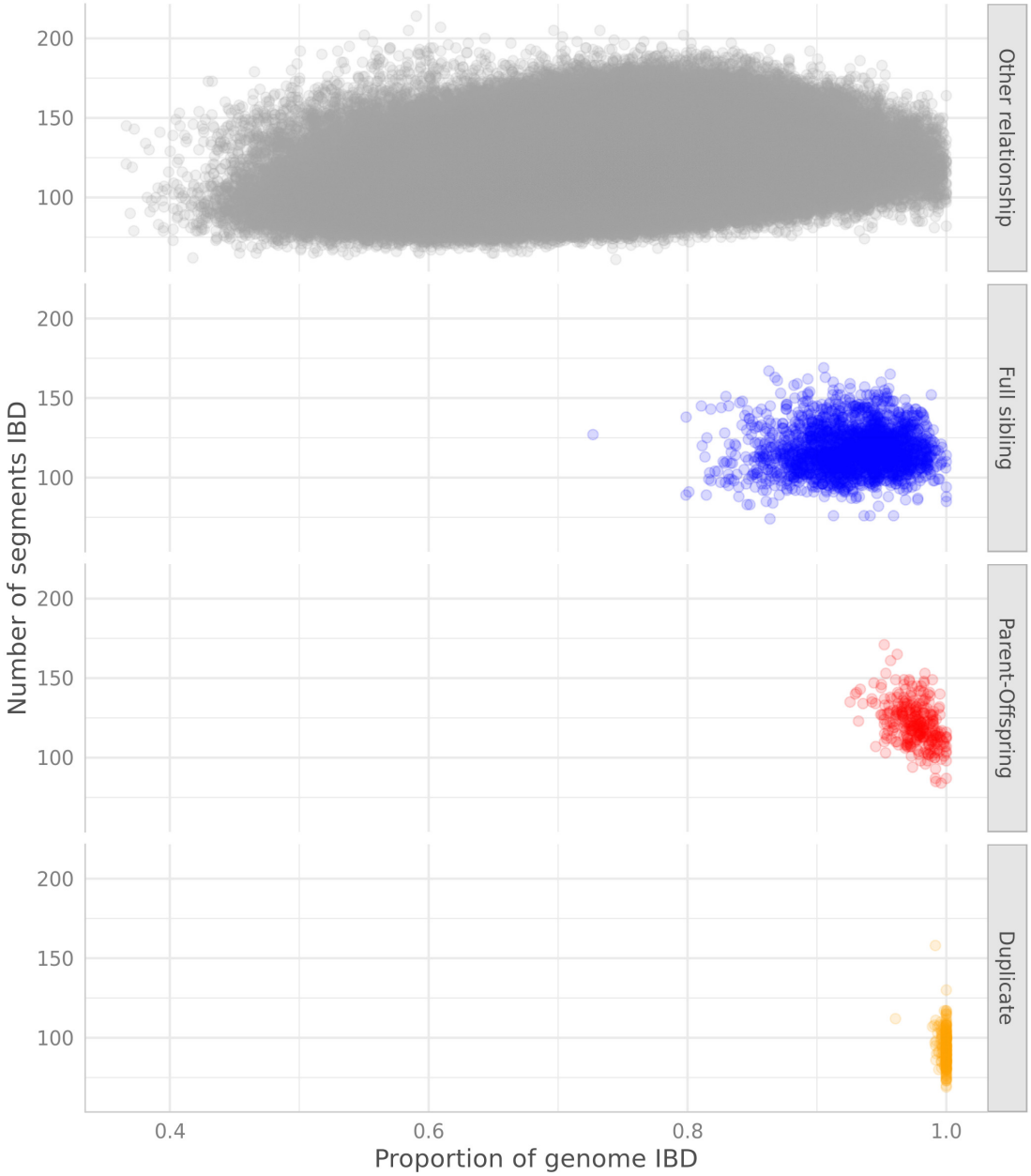

Figure 5.6: Identity-by-descent measurements (IBD) obtained from imputed genotypes compared to microsatellite-based pedigree relationships. Each dot represents the relationship between two individuals.

We calculated the proportion of loci at which two F_x individuals could not have transmitted the alleles to one another ("Mendelian errors") for the 50 most closely related individuals of each focal based on the kinship coefficient derived from IBD measures. As can be seen in Figure 5.7, this proportion decreases the fewer relationship steps are in between individuals. However, it is too high for parent-offspring (where it should be 0%), suggesting that there are still errors in the imputation, or in the pedigree relationships based on microsatellites. Finally, we plotted IBD measurements and Mendelian errors described above for parent-offspring dyads against the mean coverage between the two focals. In contrast to the number of loci and the discordance (Figures 5.3 and 5.4), these measures did not show a clear trend to improve with increasing coverage (Figure 5.8). While the number of IBD segments decreased as expected, the proportion of the genome that is IBD remained constant and the Mendelian errors even increased, which could mean that some error-prone parts of the genome are only imputed at higher coverages.

5.4 Outlook

We have demonstrated that our whole-genome imputation approach provides a high number of trustworthy genotypes. However, it will require additional work to further improve its accuracy. With the quality control measures that we laid out above, we aim to analyze the impact of several factors on the quality of the imputation. First, we will test whether the imputation improves with different genotype filtering strategies at the level of the founders. At the moment, we are filtering primarily whether loci are included based on having enough high quality genotypes, but not excluding lower quality ones. This is done because we discovered that the downstream analyses are very sensitive to the proportion of loci that were phased successfully in the founders and that proportion in turn is decreasing rapidly when loci are excluded in the F_0 and F_1. Presumably, wrong F_0 genotypes will enter the imputation pipeline because of this and consequently, we aim to test alternative strategies, such as increasing the filtering thresholds slightly before phasing and/or increasing it more strongly post-phasing. Second, we will investigate the influence of key *AncestryHMM*

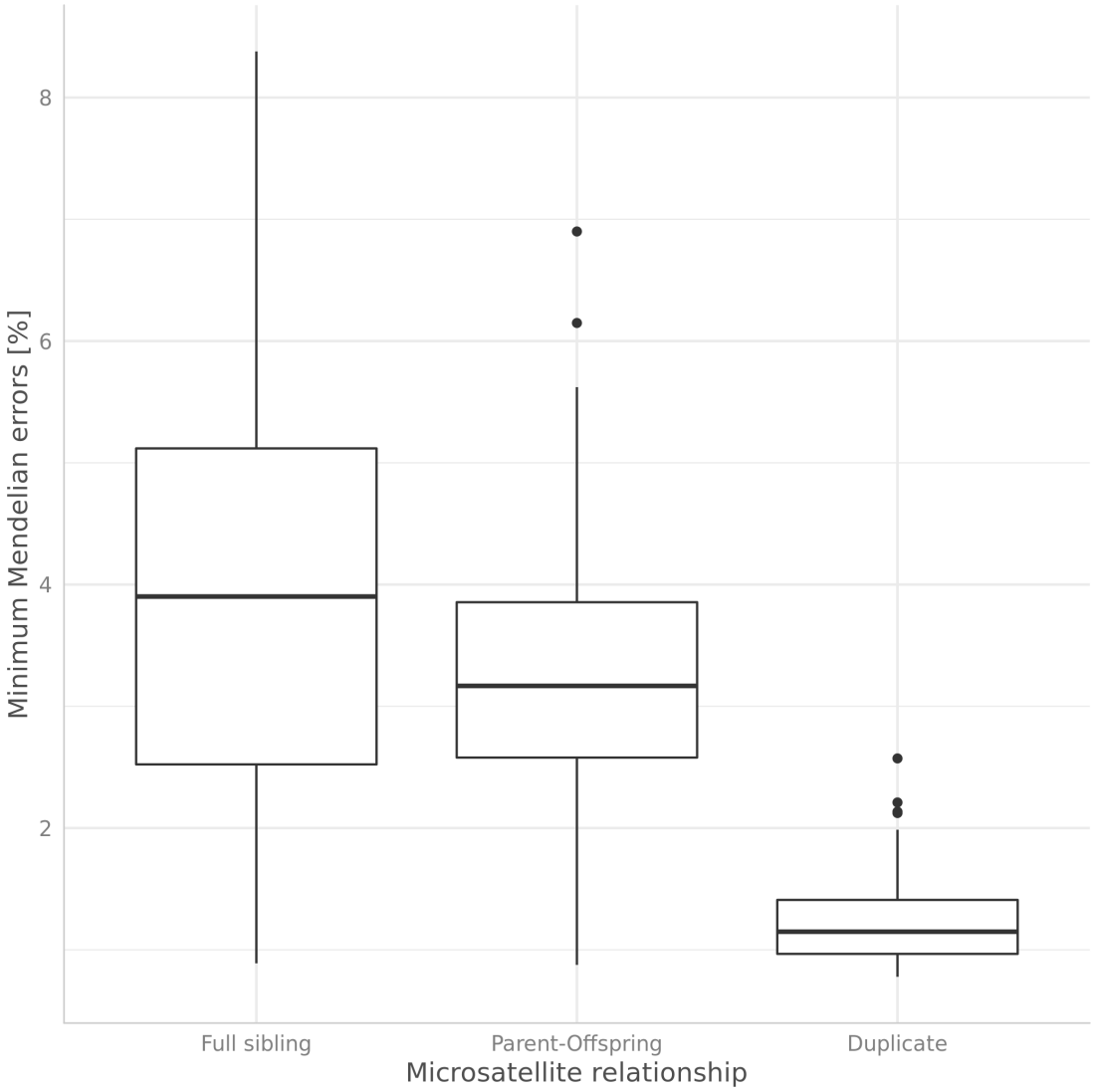

Figure 5.7: The minimum proportion of Mendelian errors based on the imputed genotypes between two mice grouped by the microsatellite relationship. Mendelian errors are computed in trios and thus the *minimum* value of all trios involving a focal duo is chosen.

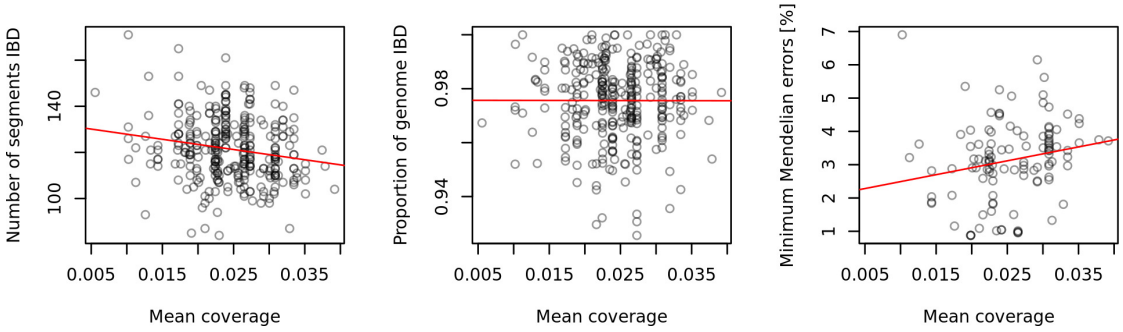

Figure 5.8: Imputation-based identity-by-descent measurements (IBD) and minimum Mendelian error rates of microsatellite-inferred parent-offspring dyads are plotted against the mean coverage between of each focal duo. Red line indicates the coefficient of a linear model.

settings on the imputation quality, namely the genotyping error rate and the number of generations that have passed for each individual since the founders. Based on early tests we assume these metrics to have minor impact, but more investigation is required. Third, our genetic map is so far based on the simplistic assumption that physical distance is a proportionate predictor of genetic distance (i.e. the likelihood of recombinations). We will analyze the ancestry probabilities to find where *AncestryHMM* appears to detect breakpoints, i.e. regions where it infers that a recombination event may have taken place. To do so, we aim to detect sudden shifts in ancestry proportions. We will then remove genotypes nearby inferred breakpoints from subsequent analyses and compare whether genotype quality increases. If it does, we can update the genetic map to reflect those apparent changes in recombination rate better. Additionally, we will analyze whether there are hotspots of errors in the imputed genotypes by searching for regions of the genome where the majority of parent-offspring-trios appear not to match consistently ("Mendelian errors"). Finally, the quality threshold we use when converting the ancestry probabilities into genotypes will allow for granular adjustments that will very likely result in better imputation, but at the cost of fewer loci.

All in all, the whole-genome imputation methodology promises to drastically be much more efficient at genotyping millions of loci than traditional methods (see Table 5.2). Once we have improved the imputation and sequenced more mice, we will be able to conduct the search for loci responsible for the variation in dispersal in this population. We will be able to conduct a very powerful anal-

ysis, as we can control for familial relationships, environmental variation, and individual condition (for example with *MENDEL* (Zhou et al. 2017)), which we expect to have substantial influences.

Table 5.2: Overview of costs and expected results of whole-genome imputation *vs.* the commercial SNP array *GigaMUGA*

Method	Informative loci	Cost per individual	Costs included
GigaMUGA	up to 7,500	circa 100 USD	Genotyping
Whole-genome imputation	$\geq$ 1,000,000	circa 15 USD	1/10,000$^{\text{th}}$ of 10x sequencing of F_0 and F_1 plus the cost of 0.05x sequencing of one F_x

5.5 Acknowledgements

Many people have made this study possible. The group of Barbara König has continuously monitored the population for 18 years. Gabriele Stichel, Sally Steinert, and Bruce Boatman were the main technicians responsible for the population. Jari Garbely extracted the DNA and genotyped the mice at microsatellite loci. Kerel Xavier Francis prepared the DNA libraries for sequencing upon which this study is based.
We thank the Swiss National Science Foundation (grants P1ZHP3_181303 to JNR, 31003A_176114/1 to BK, 31003A_160328 to AKL) and the Sloan Neuroscience Fellowship (to AB) for funding.

5.6 Author contributions

This chapter is an unpublished report on the current state of this study. All authors conceived of the study. BK founded and maintained the long-term study on which this manuscript relies. AKL created the microsatellite-based pedigree

of the study population. AB sequenced the DNA samples. JNR wrote the initial draft of the manuscript. JNR, AKL, and AB improved the manuscript. JNR conducted the study. AB contributed to the methodology.

6 Appendix

6.1 Chapter 2

Please find all supplementary material online in the published version of this chapter: https://royalsocietypublishing.org/doi/suppl/10.1098/rspb.2018.1333

6.2 Chapter 3

6.2.1 Methods

Figure 6.1 shows how the probability for the incremental (P_{inc}) and full (P_{full}) mutations changes over the course of the simulation.
Figure 6.2 illustrates the possible carrying capacities of the patches. Each patch is assigned an integer value K_p between 1 and 10 that determines the carrying capacity. Each value of K_p is equally likely to be assigned to a patch.
We wrote in the *Model* section of the paper that *t*/*t* fixate if they are not lethal. Figure 6.3 shows how quickly (within the first 100 turns) and reliably this happens, even under otherwise the least *t*-friendly conditions ($P_{\text{multi}} = 1.0$ & $M_{\text{disp}} = 0.1$).

6.2.2 Results

6.2.2.1 Verification of the simulation

We reported the variation in local density in our simulations in the paper as having a mean of 26.79 (SD=10.21). Figure 6.4 shows the distribution of the local densities.
The +/*t* frequency was found to be 0.22 (SD=0.05) under natural conditions (which equals a *t* frequency of 0.11). Figure 6.5 illustrates this distribution.

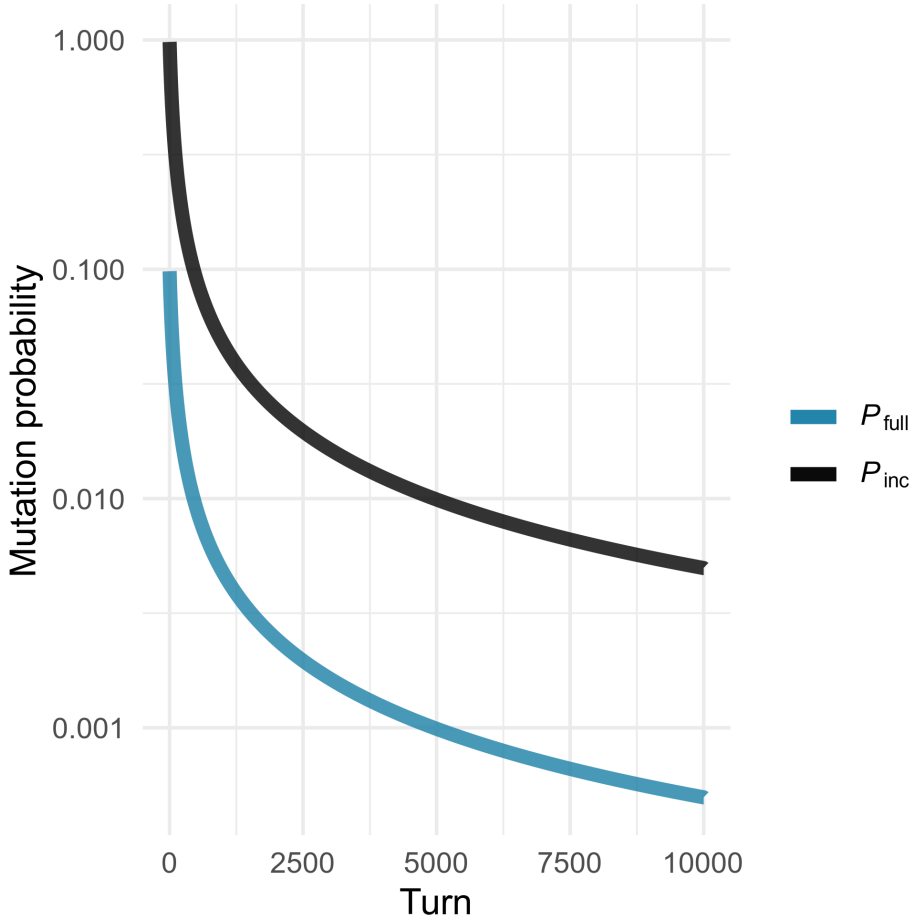

Figure 6.1: The mutation probabilities decrease over the course of the simulations. The y axis is on a logarithmic scale. P_{full} represents the probability for a locus's value to change to any value in the parameter space ("full mutation"), whereas P_{incr} represents the probability for a locus's value to increase or decrease from its current value ("incremental mutation").

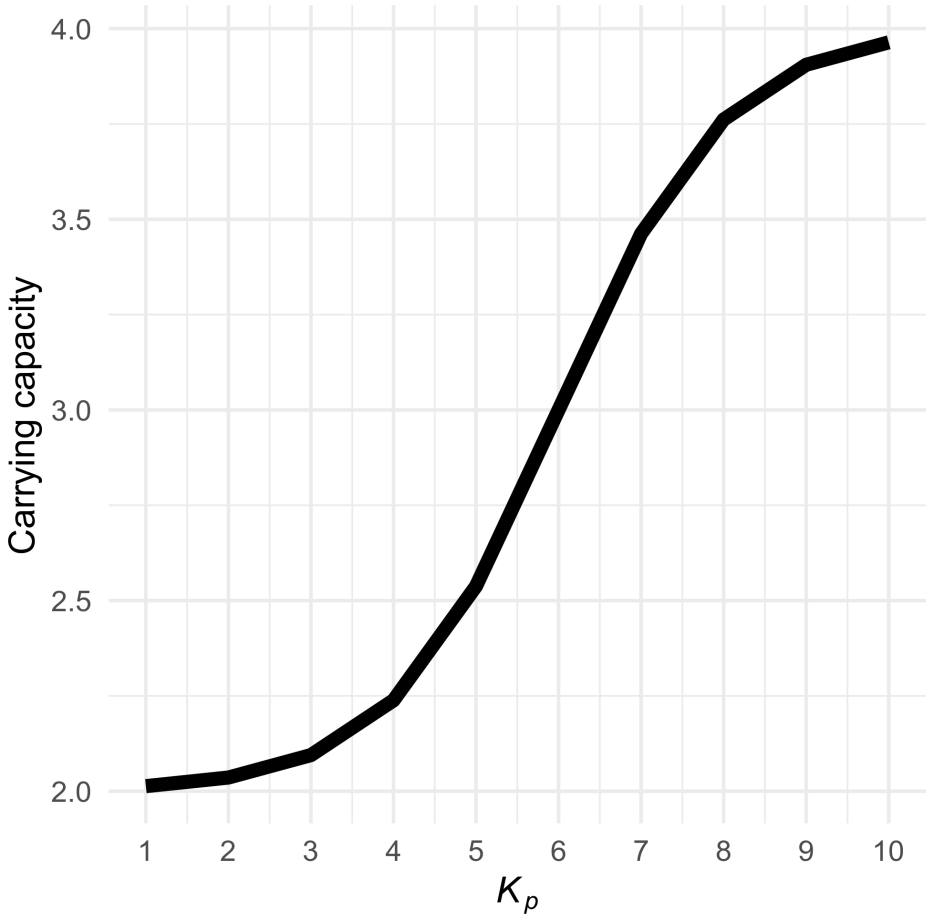

Figure 6.2: The carrying capacity in number of mice on patches of different K_p. The fraction digits represent the probability of an additional mouse surviving on that patch per turn.

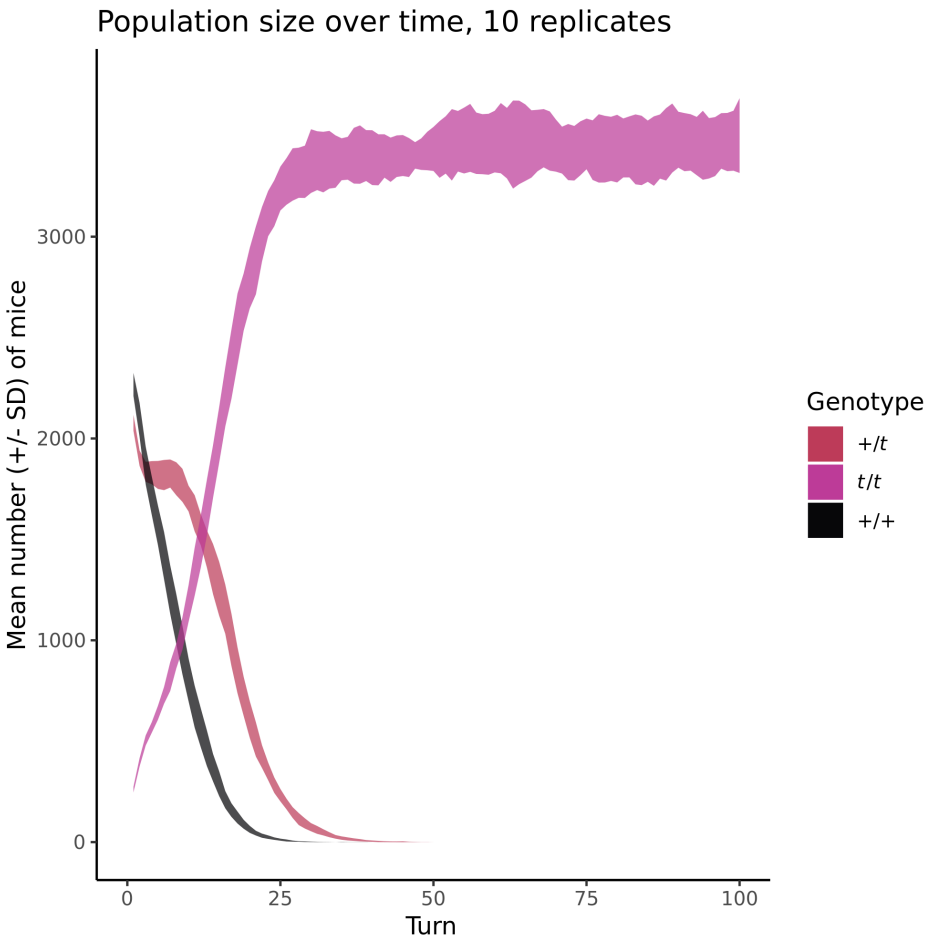

Figure 6.3: When t/t are not lethal, they quickly fixate in the population even under otherwise adverse conditions ($P_{\text{multi}} = 1.0$ & $M_{\text{disp}} = 0.1$). This figure is only showing the first 100 out of 10,000 turns.

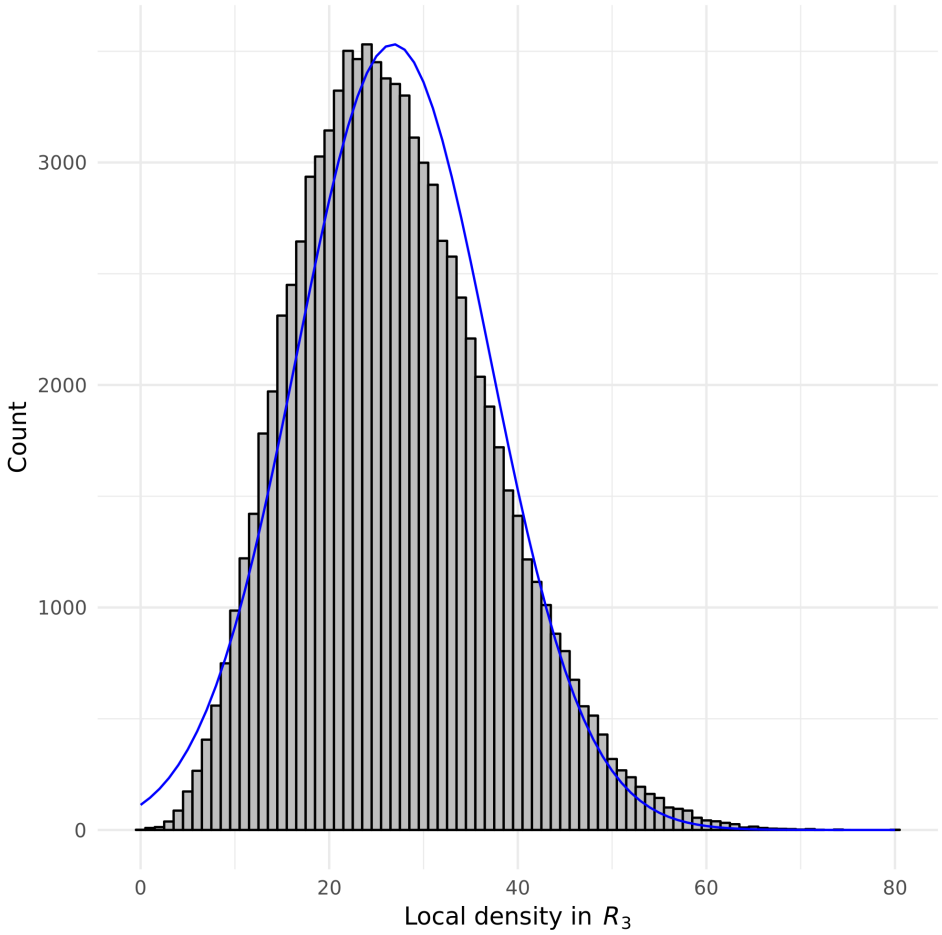

Figure 6.4: The distribution of densities in R_3 around all patches throughout the world under natural conditions ($P_{\text{multi}} = 1.0$ & $M_{\text{disp}} = 0.1$ & homozygous lethal *t*) in the final turn in 25 simulations. The blue line represents a normal distribution with the same mean, SD, and maximum value.

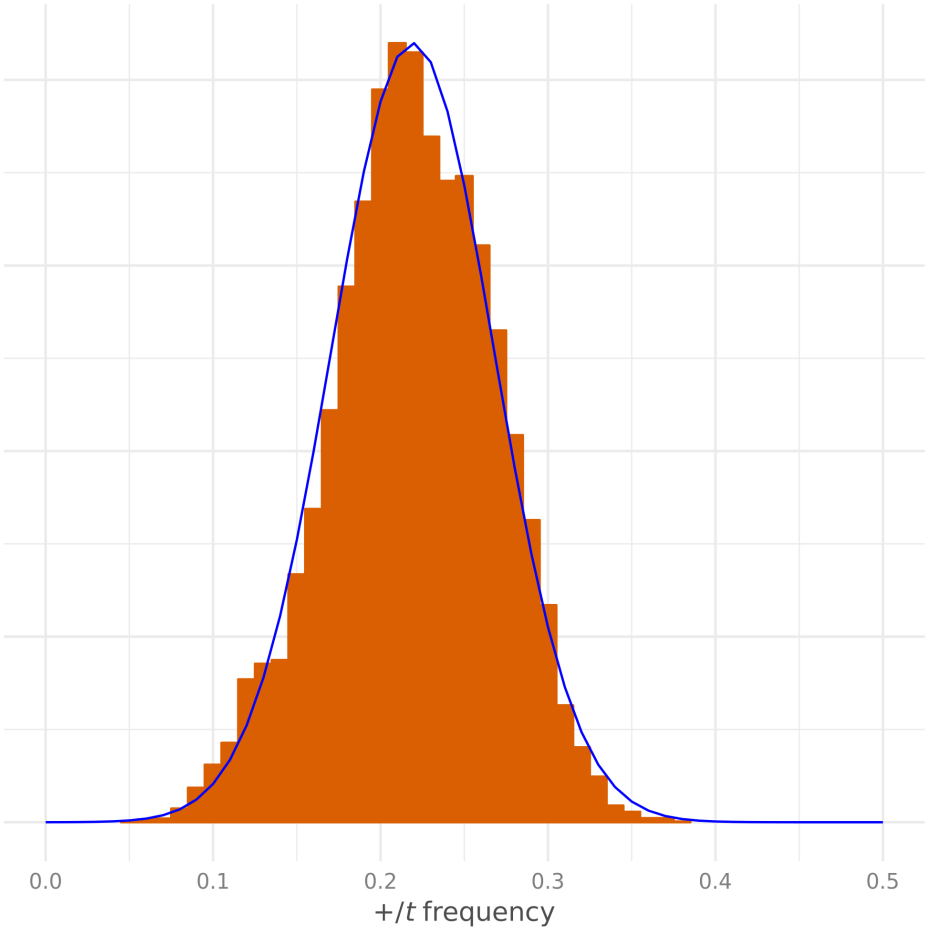

Figure 6.5: The distribution of +/*t* frequencies under natural conditions ($P_{\text{multi}} = 1.0$ & $M_{\text{disp}} = 0.1$ & homozygous lethal *t*) in turns 9000 to 10000 in 25 simulations. The blue line represents a normal distribution with the same mean, SD, and maximum value.

6.2.2.2 Additional heatmaps

In the following, we show additional figures to provide further information on how the genotypes differed in the various simulation conditions.
Figures 6.6 to 6.9 show the evolved reaction norms of the genotypes rather than the difference between them.
Figures 6.10 and 6.11 illustrate the differences between wildtype dispersal reaction norms in simulations where +/+ does or does not coevolve with +/*t*.
Finally, in Figures 6.12 and 6.13 we show that there is no reliable difference between *t*/*t* and +/+ when they are without any competing genotypes.

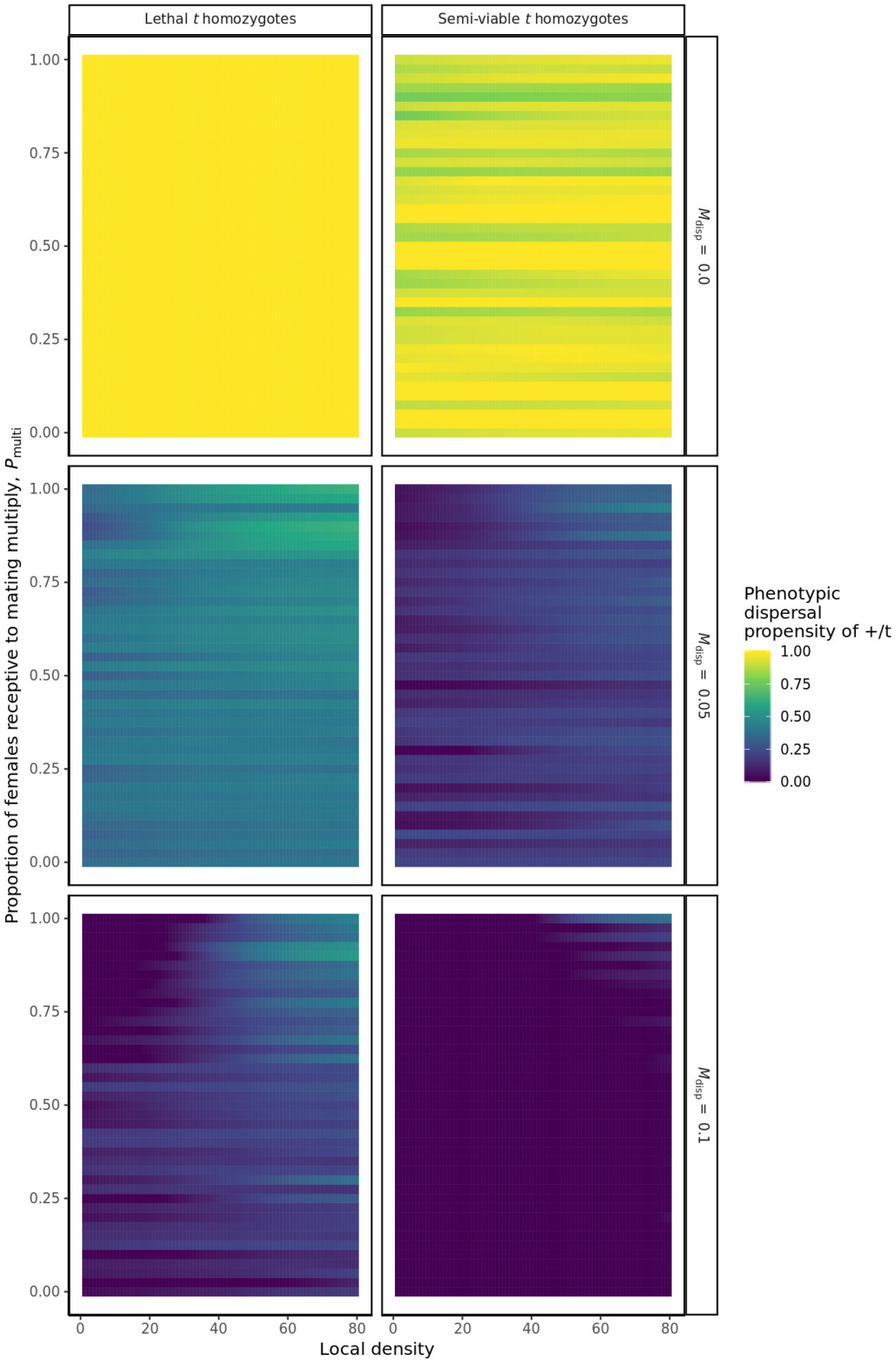

Figure 6.6: The phenotypic dispersal propensity of +/*t*.

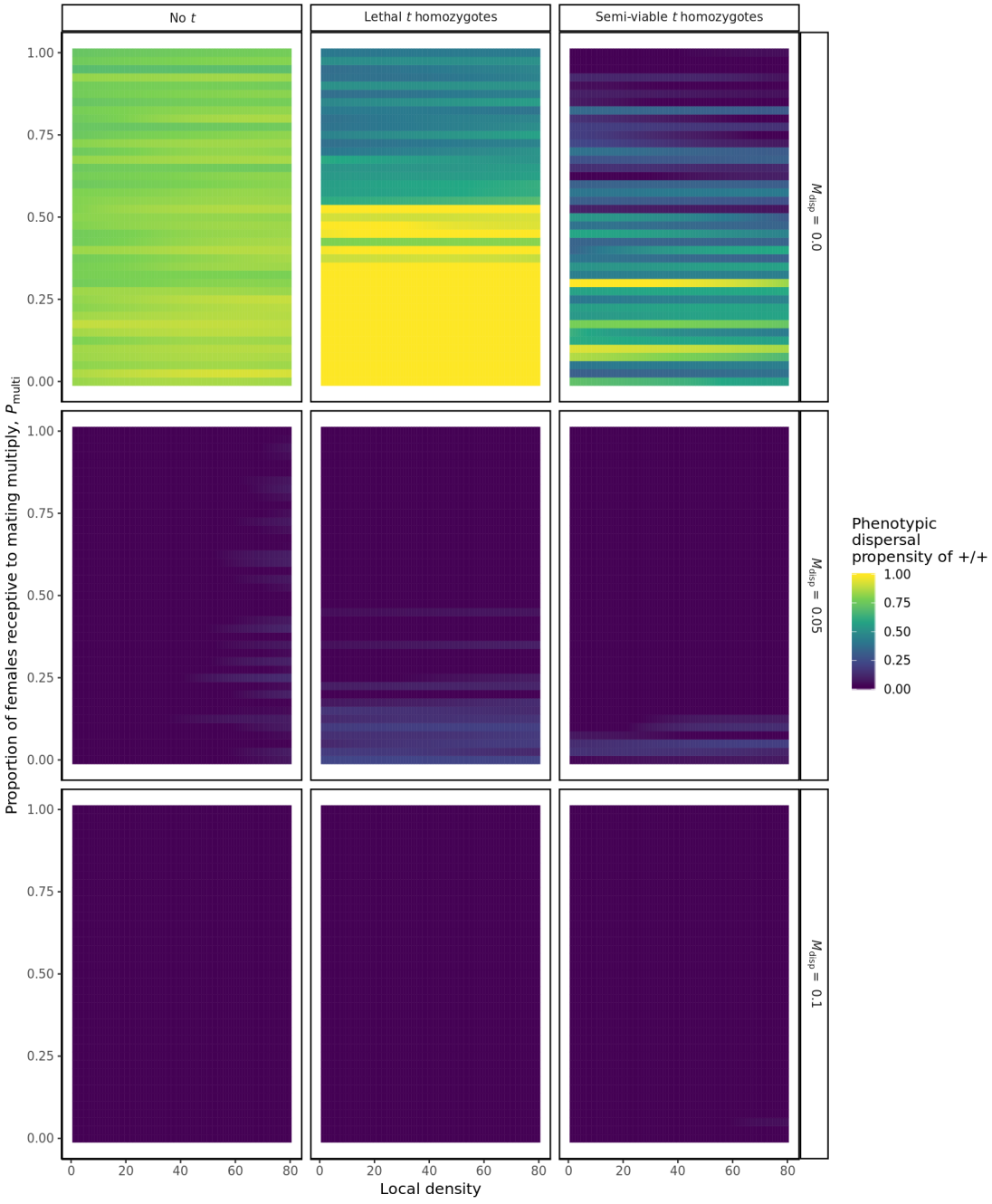

Figure 6.7: The phenotypic dispersal propensity of +/+.

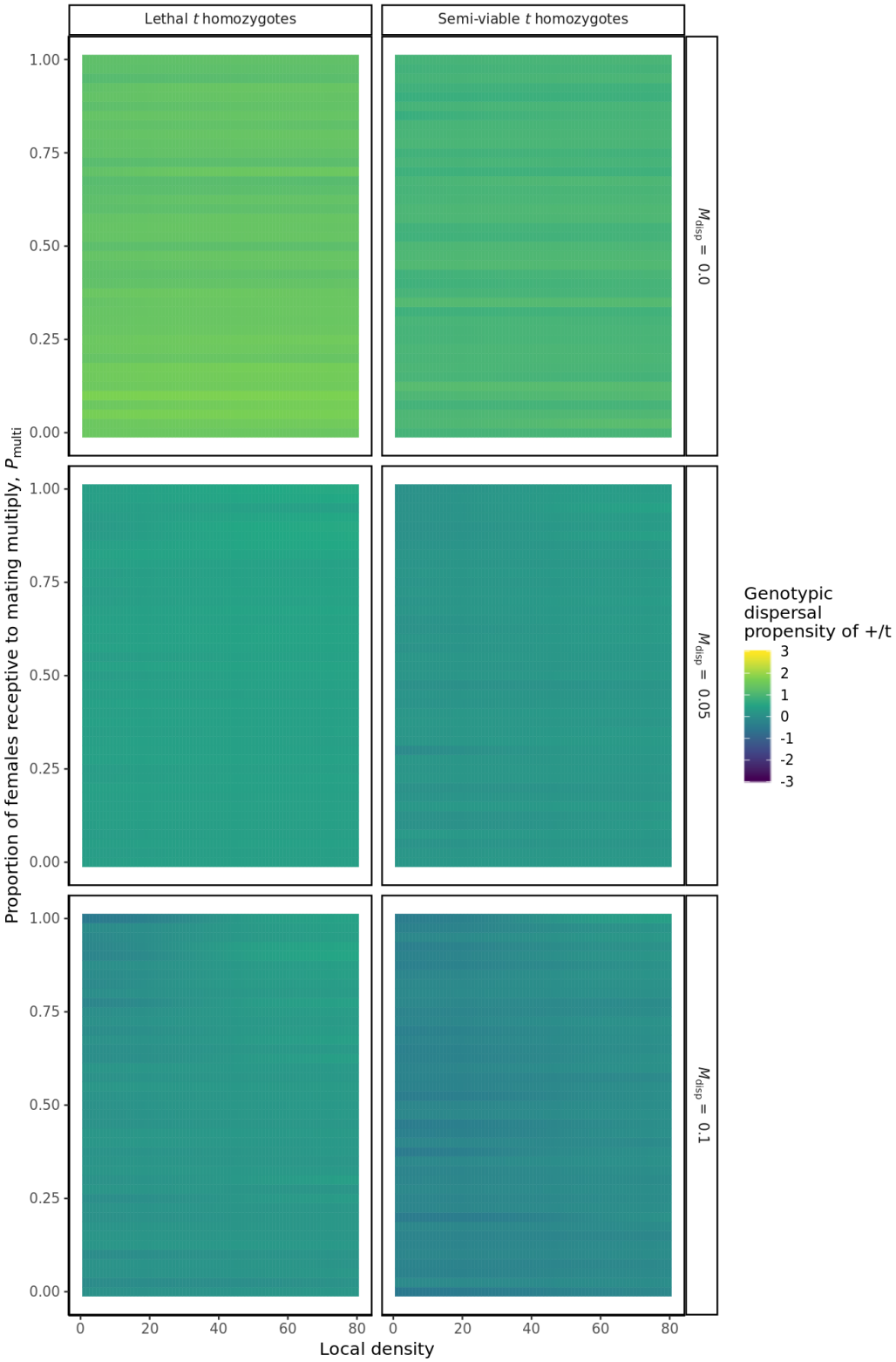

Figure 6.8: The genotypic dispersal propensity of +/*t*.

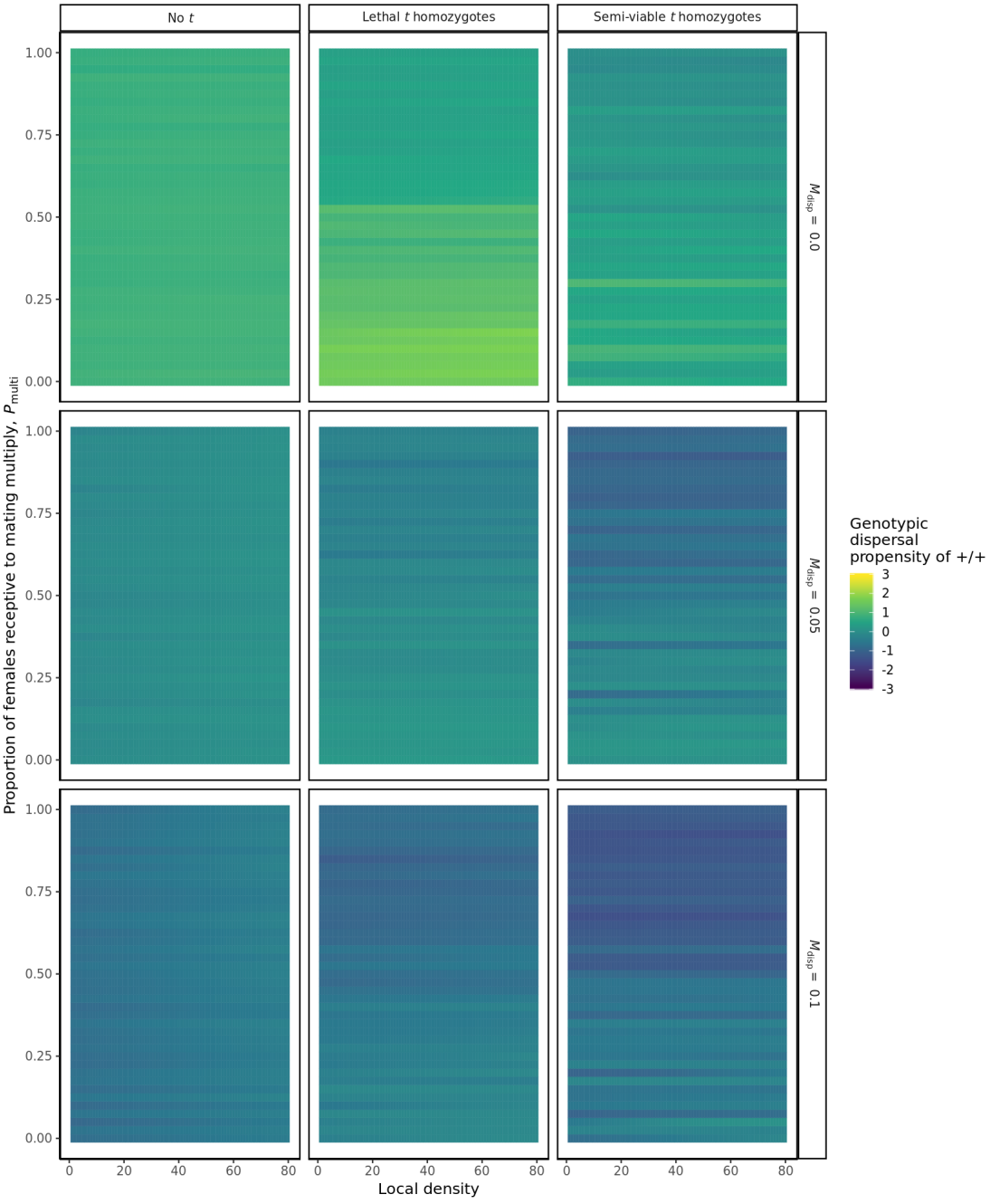

Figure 6.9: The genotypic dispersal propensity of +/+.

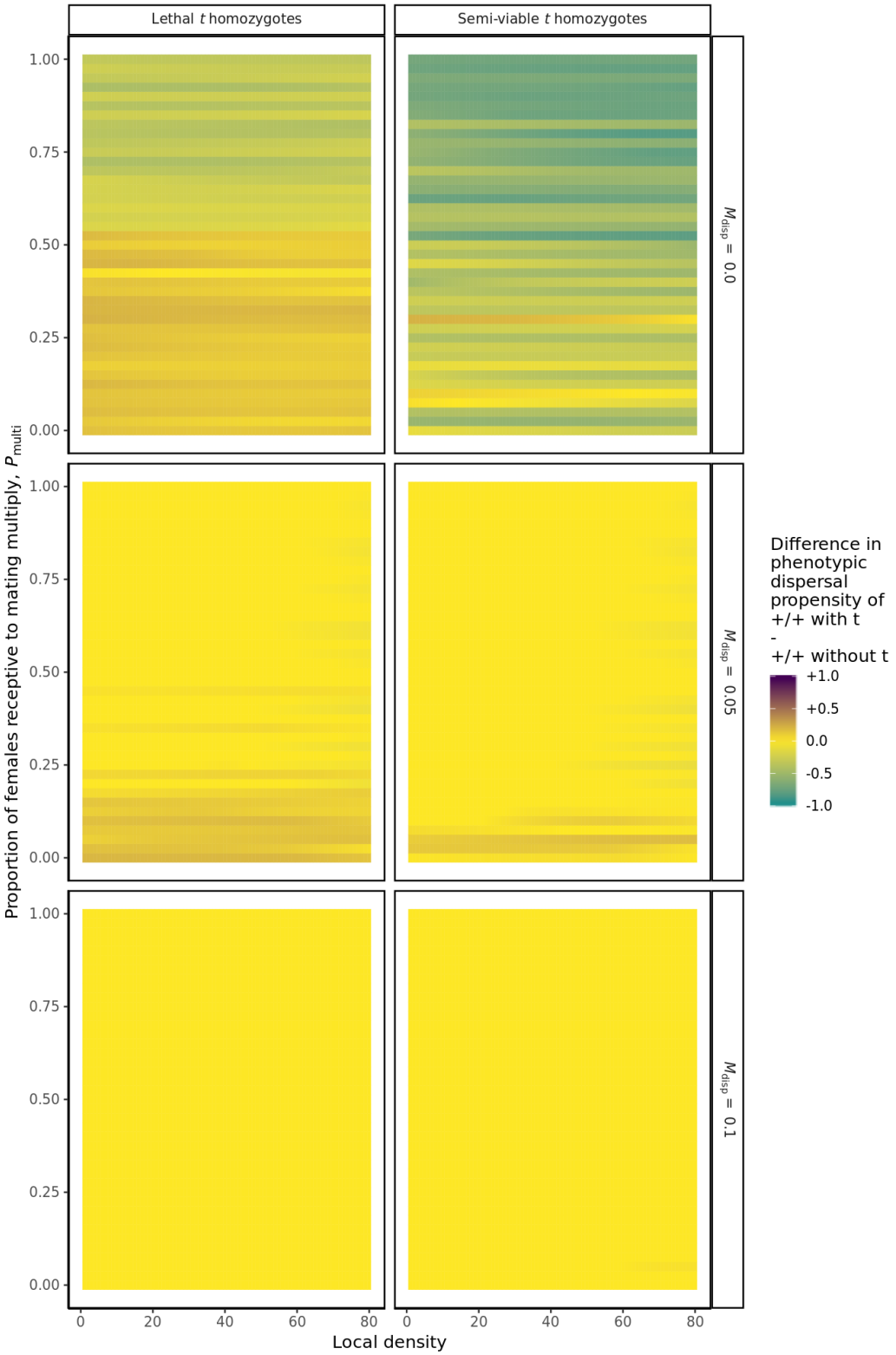

Figure 6.10: The difference in phenotypic dispersal propensity of +/+ in simulations with *t* vs simulations without *t*.

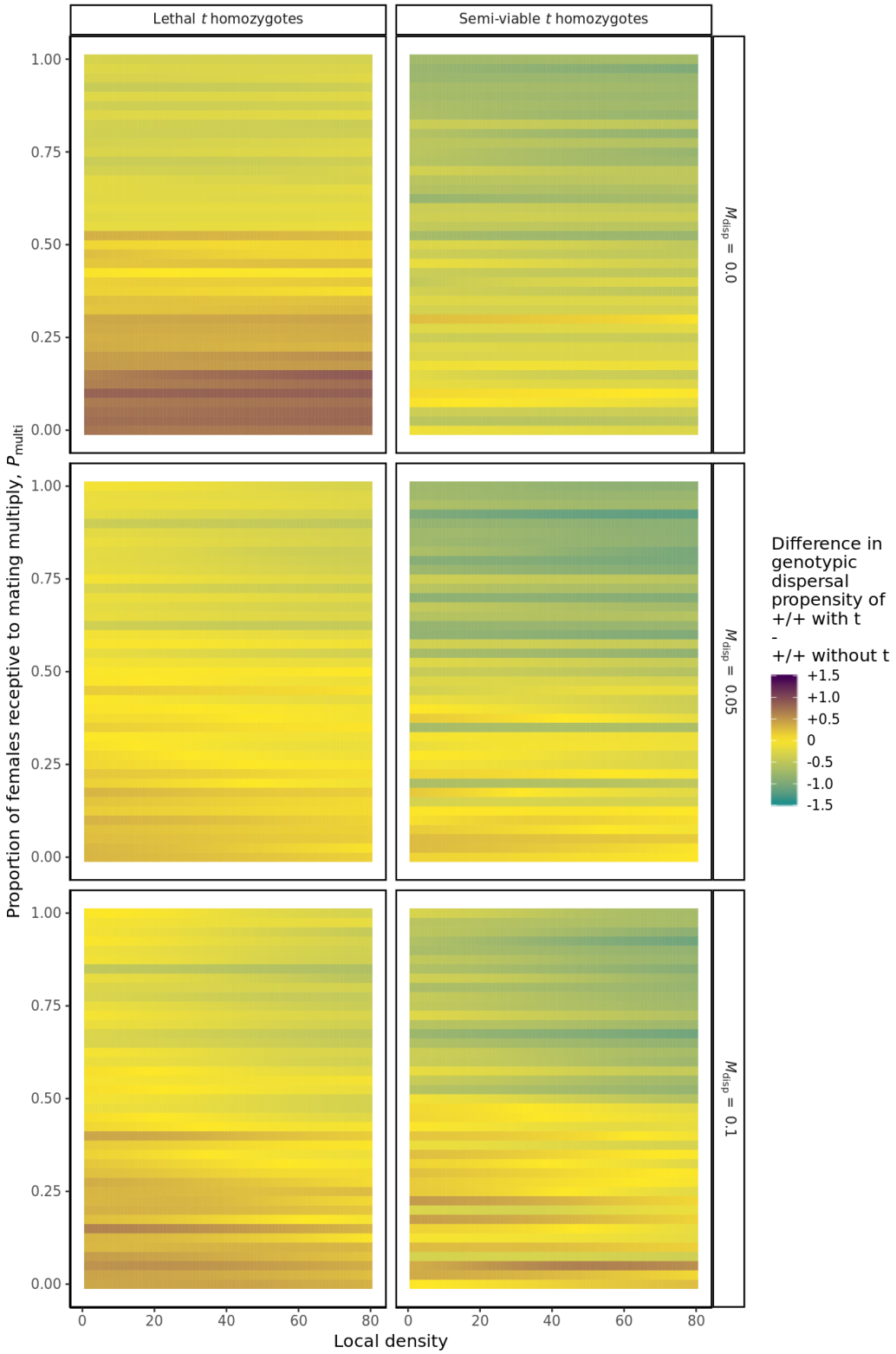

Figure 6.11: The difference in genotypic dispersal propensity of +/+ in simulations with *t* vs simulations without *t*.

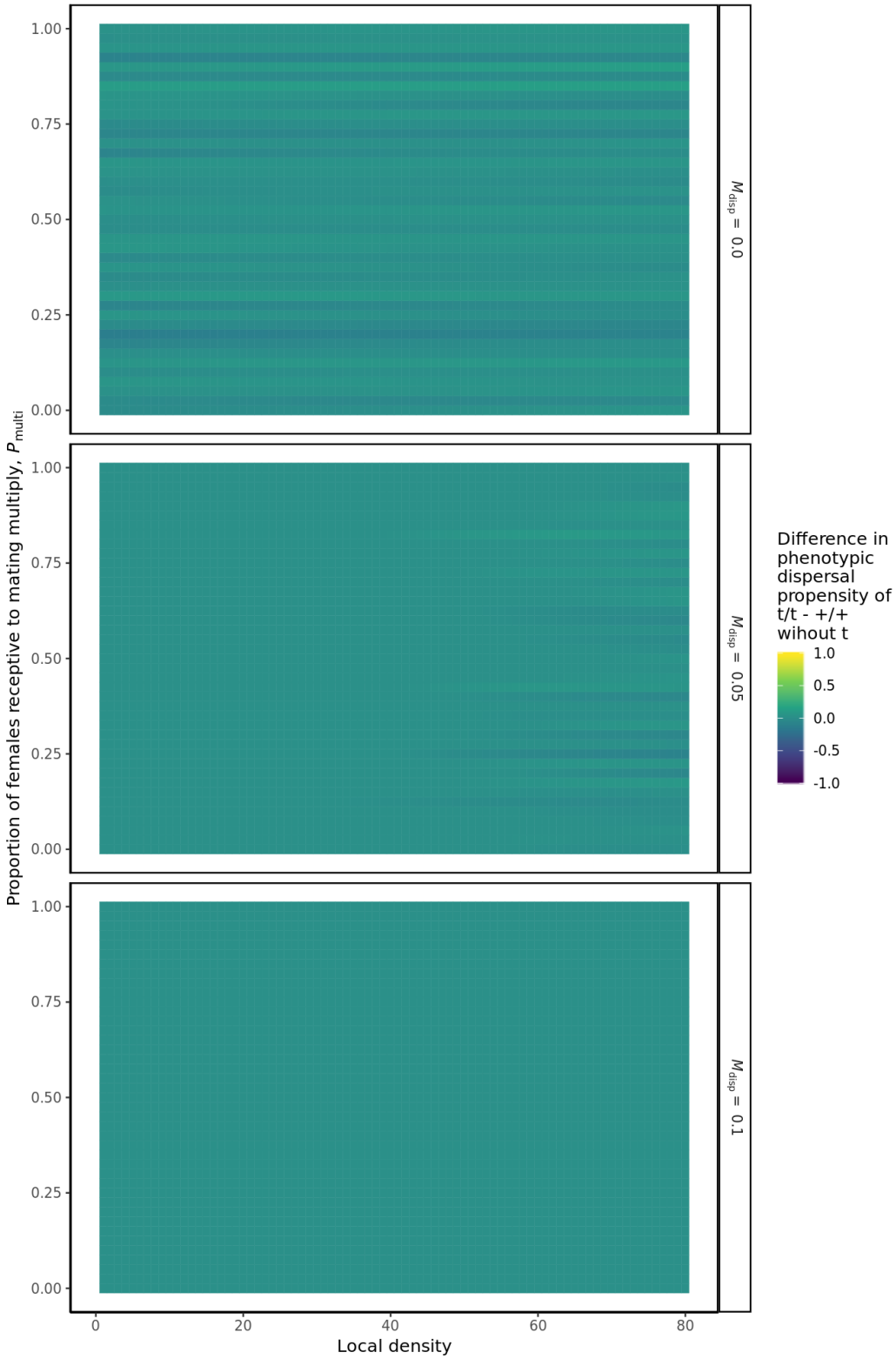

Figure 6.12: Difference in phenotypic dispersal propensity of *t*/*t* - +/+, the latter in simulations without *t*.

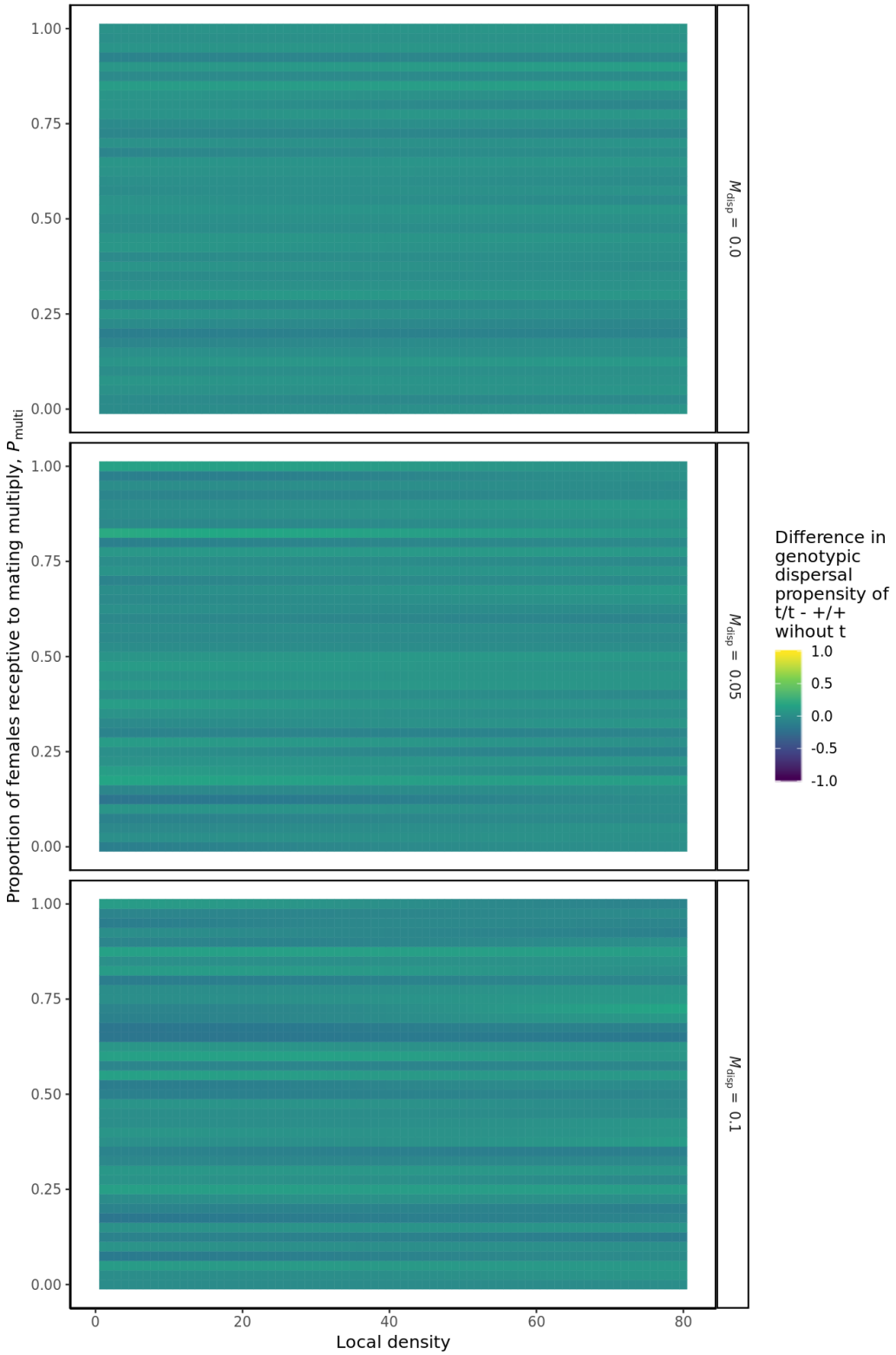

Figure 6.13: Difference in genotypic dispersal propensity of *t*/*t* - +/+, the latter in simulations without *t*.

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